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Biodiversity and Aquaculture - for Sustainable Development

Proceedings of the Twenty-fifth UJNR Aquaculture Panel Symposium
Yokohama, Japan
October 16 - 17, 1996

Edited by Masanori Azeta, Kazufumi Takayanagi,
James P. McVey, Paul Kilho Park and B. Jane Keller

Under the U. S. -Japan Cooperative Program in Natural Resources (UJNR)

National Research Institute of Aquaculture
Fisheries Agency

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Panel Chairmen:
Yukio Uekita, Japan
James P. McVey, United States

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PREFACE

A joint United States and Japanese panel on aquaculture was formed in 1969 under the United States-Japan Cooperative Program in Natural Resources (UJNR). The panel currently includes specialists drawn from the government agencies, laboratories, industry, academia, some of which are parts of the U.S. Sea Grant Program in the field of aquaculture. Charged with exploring and developing bilateral cooperation, the panel has focused its efforts on exchanging information related to aquaculture which could be of benefit to both countries.

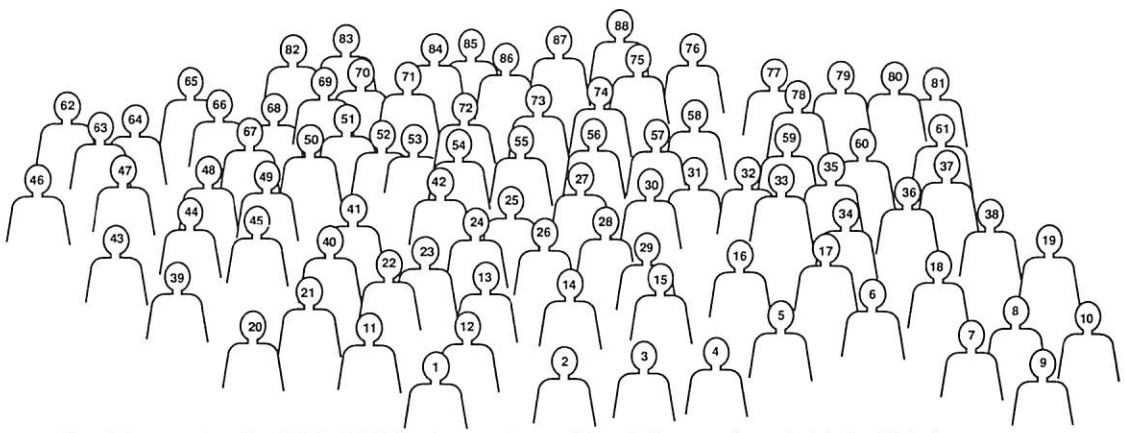
The UJNR was begun during the Third Cabinet-Level Meeting of the Joint United States-Japan Committee on Trade and Economic Affairs in January 1964. In addition to the aquaculture panel, current subjects in the program include desalination of seawater, toxic microorganisms, air pollution, energy, forage 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 include: Increased communication and cooperation among technical specialists; exchanges of information, data, and research findings; annual meetings of the panels, a policy-coordinative body; administrative staff meetings; exchanges of equipment, materials, and samples; several major technical conferences; and beneficial effects on international relations.

The 25 th US - Japan Aquaculture Panel Symposium was held in Yokohama, Japan, from October 16 to 17, 1996, focusing on "Biodiversity and Aquaculture - for Sustainable Development". Two special sessions were also held during a field trip to Tsukuba from Yokohama: (1) Discussions on the wise usage of coastal waters in Kanagawa Prefecture held in Yokosuka on October 18 and (2) Discussions on the enhancement program of Japanese flounder in Ibaraki Prefecture held in Hasaki on October 22. The symposium was organized by program chair Nagahisa Uki and other UJNR Aquaculture Panel staff members of the Japanese side. More than 30 papers were presented in the symposium. Twenty-six of them have been accepted for this Proceedings. Editorial work has been assisted by the NRIA staff.

Chairmen:
Yukio Uekita, Japan
James P. McVey, United States

This Supplemental Bulletin is published by National Research Institute of Aquaculture (NRIA), of the Fisheries Agency in Japan as a symposium volume of the United States-Japan Cooperative Program in Natural Resources (UJNR) Aquaculture Panel Meeting. It was edited by the Japanese UJNR Aquaculture Panel in accordance with the agreement between US and Japan Panel at the 23rd business meeting that the proceedings should be produced by the nation which hosts the meeting. The proceedings before the 20th symposium edited by the US UJNR Aquaculture Panel have been published as the series of NOAA Technical Report (NMFS). It has been renamed as UJNR Technical Report since the 22nd symposium in Homer, Alaska, 1993.



Participants in the 25th UJNR Aquaculture Panel Symposium held in Yokohama, October 1996

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39. Takeshi MURAI
40. Kazunori FUJII
41. Koji YOKOKAWA
42 Ryo KIMURA
43. Tateki MIYAMOTO
44. Kazuo TABATA
45. Katsunori KIMOTO
46. Masayoshi UNO
47. Hiroshi KAWAI
48. Takuji UCHIDA
49. Tadashi ODA
50. Hisashi OKABE
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55. Theodore I. J. SMITH
56. A. J. PAUL
57. Katsuyuki SASAKI
58. Cheng Sheng LEE
59. Akio SHIMIZU
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61. Ryozo KAMINOKADO
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63. Masahiro HAYASHI
64. Takeshi HARA
65. Goro YOSHIDA
66. Seiichi KANAMAKI
67. Yoshiharu NAKAMURA
68. Shunji SUZUKI
69. Robert R. STICKNEY
70 Noboru AISHIMA
71. Hans ACKEFORS
72. Bruce J. BARBER
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82. Yoshizo ITOH
83.
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86. William R. HEARD
87. Yo YAMASHITA
88. John M. MILLER

Contents

Bull. Natl. Res. Inst. Aquacult., Suppl. 3 : 1－1 (1997)

PURPOSE AND PERSPECTIVE OF THE SYMPOSIUM

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The role of fisheries as an essential source of healthy animal protein for humanity has become prominent in recent years, especially a rapid increase in the world population demands its effective, sustainable utilization. Furthermore, although capture fisheries may have reached their upper limit globally, aquaculture, including sea-farming, has a potential to double the present yield and its development is highly expected.

Concurrently, one of the most urgent environmental problems for humanity's sustainable development is the loss of biodiversity, the richness of life that has evolved in our planet. In order to achieve sustainable development of aquaculture, we should consider the environmental problem especially concerning biodiversity. There are regional, national, and international differences in the relationship between humanity and nature in accordance with the differences in natural environmental features, culture, and history. There are various concepts in the conservation of nature or the environment according to these regional, national or international attitudes. In Japan, we have lived in a narrow island country surrounded by the most fertile seas in the world and have developed a distinctive culture and life style acclimated to and confronted by natural phenomena. Our ancestors have actively engaged in fishing over 6000 years and efficiently utilized various gifts from the sea, including not only benthic sea urchins and shells but also ocean-migrating tuna and whales. Today, we are living affluently and appreciating a variety of foods. We obtain 40% of animal protein from many kinds of fisheries products.

In other words, we have established an intimate relationship with the sea through fishing and recreation. Consequently, we have been gradually changing the nature of the sea. For us, the concept of conservation of the sea does not simply imply the conservation of original pristine wilderness without human impact. Our concept implies the conservation of marine environmental conditions which will sustain the relationship between humanity and the sea for a reasonable length of time.

Through evolution during a long geological age, the marine ecosystem has been composed of exquisite works of tremendous organisms. They efficiently and steadly sustain the system and give us immense gifts from the sea, such as foods, medicines, industrial products, amenities, and also they have inexhaustible unexploited potential. In order to enjoy the continuous services from the fertile sea, healthy biodiversity of the marine ecosystem should be maintained.

However, the effects of human activities, such as destruction and extinction of healthy fishery habitat, water and sediment pollution, and introduction of non-native species, are drastically accelerating, and conservation of biodiversity has become one of the most urgent issues in our day. It is our duty to clarify the role of biodiversity at the gene, species, and ecosystem levels for the sustainable development of aquaculture, as well as to exploit the methods and policies to conserve biodiversity in the sea.

In this symposium we would like to discuss the perspectives of aquaculture from the long-range and global viewpoint to achieve sustainable development of aquaculture in Japan and the United States. Discussion by scientists of both countries, which have differences in cultural and societal infrastructures, will promote the comprehensive and universal understanding for relationships between sustainable development of aquaculture and biodiversity. We also endeavor to search for a mutually beneficial avenue in the cooperative flounder research and other possible future research in aquaculture to arrive at a joint five-year cooperative plan for the UJNR Aquaculture Panel.

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RELATIONSHIPS BETWEEN RED TIDE OCCURRENCE, PHYTOPLANKTON DIVERSITY, AND VERTICAL STABILITY OF SEAWATER IN EMBAYMENTS OF JAPAN

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ABSTRACT

The relationships between red tide occurrences of *Gymnodinium mikimotoi*, phytoplankton diversity, and the vertical stability of seawater were examined on the basis of six-year observations of the phytoplankton composition in Gokasho Bay and for five years in Uranouchi Bay, Japan. *G. mikimotoi*, which is a K-strategist, appears by spring and grows to form red tides in June-August. The cell density of this species was found to increase during periods of vertical stability of seawater and to decrease during periods of vertical turbulence. Similarly, the phytoplankton species diversity index (SDI) appeared to increase as the vertical structure of seawater became stable. Further, the SDI decreased during the occurrence of *G. mikimotoi* red tides. Thus, turbulence of seawater is considered to renew the environment for phytoplankton. This may contribute to minimize the occurrence of red tides and to maintain high SDI levels. In this respect, high densities of aquaculture facilities which may interfere with the flow of seawater should be avoided.

INTRODUCTION

Phytoplankters are the principal primary producers of most marine ecosystems. Fish and shellfish feed on them directly or indirectly through the food chain and the diversity of this fauna present in the sea is dependent on the species diversity of phytoplankton since different faunal species have different feeding habits. In embayments around Japan, red tide outbreaks by nuisance phytoplankton species have increased with the eutrophication levels of the seawater. The red tides caused by flagellates are, in most cases, monospecific. Red tides by these flagellate species often involve not only the death of many marine animals, but also a decrease of the phytoplankton diversity in the surrounding waters. Therefore, by analysis of the phytoplankton community including red tide species, useful information can be obtained so that the effects of red tides can be minimized when planning aquaculture for those embayment areas.

The aim of the current study is to elucidate the significance of the phytoplankton diversity and the vertical stability of seawater under the occurrence of a *Gymnodinium mikimotoi* red tide. Long-term survey data of the phytoplankton communities of Gokasho Bay and Uranouchi Bay are given as examples.

ECOLOGICAL FEATURES OF THE RED TIDE ORGANISM, *GYMNODINIUM MIKIMOTOI*

We have observed the species composition of the phytoplanktons in both Gokasho Bay and Uranouchi Bay (Figure 1) every 7-14 days at five depth layers (0, 2, 5, 10m, and 1m above the bottom). In the present study, the results obtained from 1990-1995 in Gokasho Bay and also from 1991-1995 in Uranouchi Bay are compared. Seawater temperature and phytoplankton number counts were carried out as described in Honjo et al.[1] During the investigations, red tides by *G. mikimotoi* were observed around the monitoring stations in 1990 and 1994 at Gokasho Bay, and in 1991 and 1994 at Uranouchi Bay.

As shown in Figure 2 A, *G. mikimotoi* cells had already appeared in April 1990 at Gokasho Bay. After repeated fluctuations, the mean cell density of *G. mikimotoi* in the water column reached about 1,000 cells ml^{-1} in early August. Similarly, in other cases (Figure 2 B-D), this species appeared in spring and caused red tides in summer. It is noteworthy that this species was present in the water column for a long period prior to the outbreak of the red tide. Both in situ and in vitro studies showed that this species grows rather moderately (maximum growth rate=1 div. day^{-1}), and requires low light levels for its

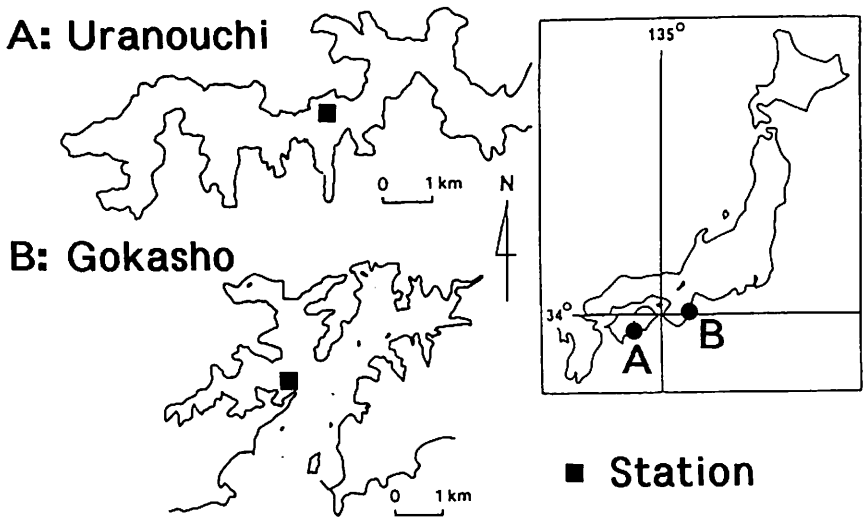

Figure 1. Stations used in this study for monitoring phytoplankton and seawater temperature.

growth.[2-5] On the other hand, two raphidophycean red tide species *Chattonella marina* and *Heterosigma akashiwo* show high growth rates during early growth phase.[6,7] In the natural habitat, *G. mikimotoi* shows clear diurnal vertical migration, moving to as deep as 25-30 m at night[8]. Furthermore, this species grows at a depth deeper than 2 m during the early growth phase[9]. In this study, high cell densities of *G. mikimotoi* cells were frequently observed at 5-m depth during its early growth phase and then appeared in the upper 0-2 m depth layer during the red tide occurrence. These features are advantageous for the growth of this species since it can utilize the higher nutrient contents of the deep waters, and also avoid the more variable physical conditions of the surface water. Considering these physiological and ecological characteristics, *G. mikimotoi* is a K-strategist which grows moderately and is highly competitive[10] among the microalgae populations. On the other hand, *H. akashiwo* and *C. marina* have the features of an r-strategist compared to *G. mikimotoi* as predicted from their high growth rates.

Thus, *G. mikimotoi* appears by spring but does not form red tide until conditions are appropriate for its growth.

GYMNODINIUM MIKIMOTOI RED TIDES AND THE VERTICAL STABILITY OF SEAWATER

Increases of abundance of *G. mikimotoi* appear to occur during periods when the water column is stable in terms of the difference between the surface and bottom seawater temperatures (Figure 2 A-D). The growth of this species was apparently suppressed by vertical turbulence of the seawater and the growth of this species recovered with the renewed formation of a stratified water column. This pattern is clearly shown in 1990 at Gokasho Bay (Figure 2 A) and in 1991 at Uranouchi Bay (Figure 2 C), respectively. Clear relationships between the growth of this species and the vertical stability of seawater were found. Thus, the stability of seawater is considered to be an important factor for the development of red tides by *G. mikimotoi*.

RELATIONSHIPS BETWEEN RED TIDE OCCURRENCE, SPECIES DIVERSITY OF PHYTOPLANKTON AND SEAWATER TURBULENCE

Generally, with the succession of phytoplankton an increase of the species diversity of the phytoplankton community also occurs during the period of vertical stability of seawater with intermittent mixing[11,12]. This tendency was also found in the present study. Phytoplankton species diversity index (SDI) was determined using the Shannon-Wiener function[13]. In both bays investigated, more than 40 species of phytoplankton (determination limit, > 0.1cells/ml) were recorded. Although for some genera the species identification was difficult and in these cases each genus was regarded as one species. Therefore, the SDI calculated here is not Shannon-Wiener's index in the strict meaning ; however, it allows an appreciation of the general features of the fluctuations of phytoplankton diversity in each bay. As shown in Figure 2, the SDI seems to increase during the stable periods of the water column. However, in 1990 at Gokasho Bay, the SDI decreased in late July although the vertical structure of the water column was considerably stable (Figure 2 A). At this time, *G. mikimotoi* cells grew to form a red tide. Apparently, red tides of this species decrease the species diversity level of the phytoplankton. Such relationships between SDI, red

tide outbreak, and the vertical stability of seawater can be noticed in other cases (Figures 2 B-D). Thus, turbulence of seawater is considered to affect both the species diversity of phytoplankton and to contribute to the decrease of cell density of the red tide organism. This physical event of water column mixing renews the ambient environmental condition for phytoplankton succession. With renewed water column stability, phytoplankton succession advances and SDI increases. In eutrophicated embayments, however, noxious flagellates grew to form red tides which decreased the phytoplankton diversity.

The changes of SDI throughout the investigation are shown in Figure 3. SDI was highly variable in both bays. However, the annual mean value of SDI did not vary markedly both between years and between bays (Table 1). It seems that the annual mean SDI was not so affected by the occurrence of *G. mikimotoi* red tide.

The repetition of periods of turbulence and of stability of the seawater structure may support species diversity of phytoplankton and permit many species to grow in embayments.

CONCLUSION

The present study shows that intermittent physical turbulence of seawater might minimize red tide occurrences as well as minimize the effects of eutrophication, and also maintain the high phytoplankton diversity in the embayments. In this respect, intensive aquaculture where a high density of artificial structures occur should be avoided since it may interfere with the flow of seawater.

Table 1. Annual mean phytoplankton species diversity index in Gokasho and Uranouchi Bays

Bay\Year	1990	1991	1992	1993	1994	1995	Average
Gokasho	0.473	0.389	0.380	0.445	0.445	0.555	0.448
Uranouchi		0.359	0.405	0.468	0.396	0.557	0.437

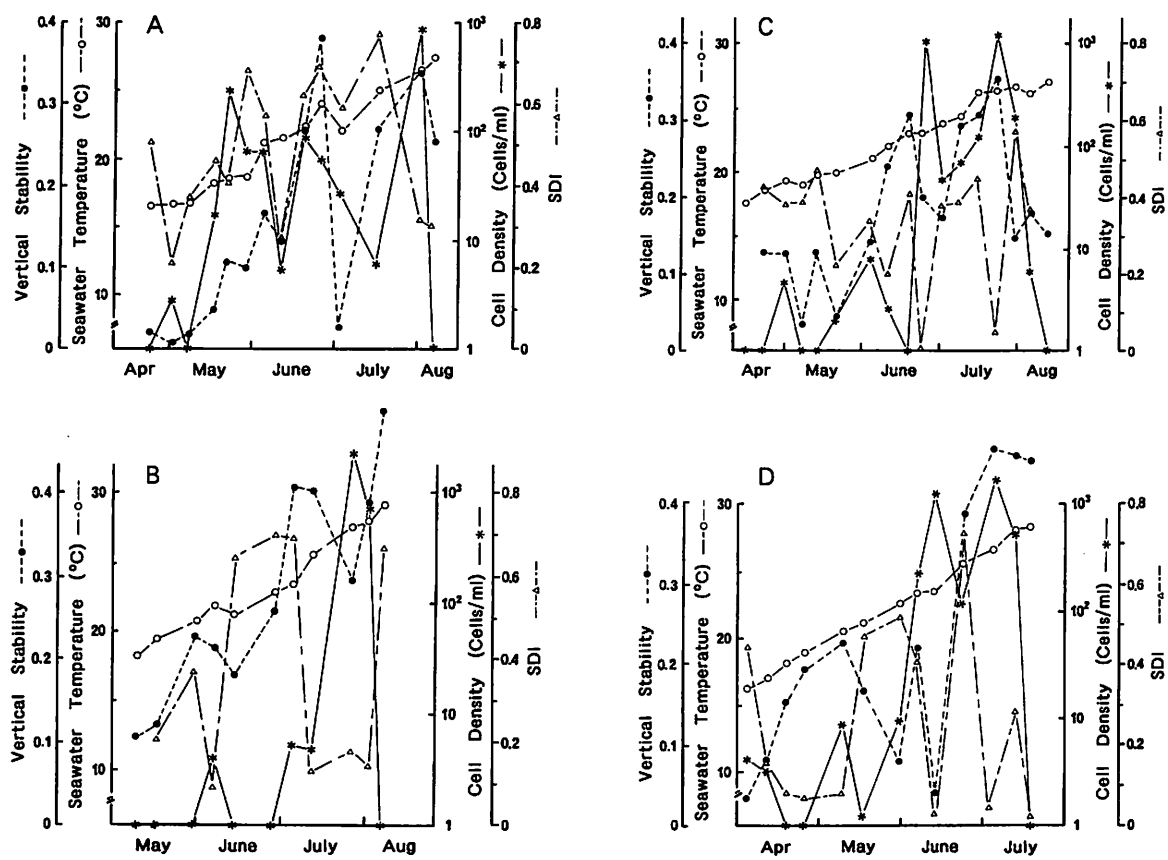

Figure 2. Relationships between the cell density of *Gymnodinium mikimotoi*, phytoplankton species diversity index (SDI) and the vertical stability of the water column. The difference of temperature between the surface and bottom seawater was divided by the depth and used as vertical stability of the water column. Seawater temperature is mean value of the water column.
A: Gokasho Bay in 1990, B: Gokasho Bay in 1994, C: Uranouchi Bay in 1991, D: Uranouchi Bay in 1994.

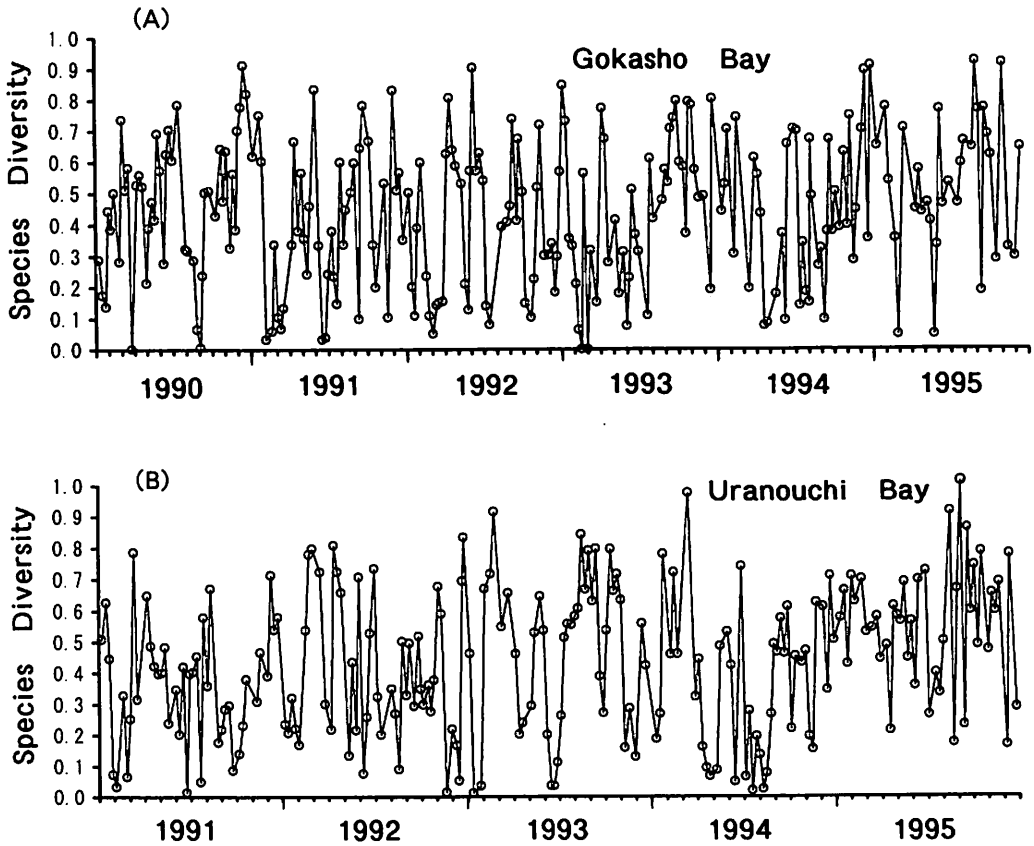

Figure 3. The changes of phytoplankton diversity in Gokasho Bay (A) and Uranouchi Bay (B).

ACKNOWLEDGMENTS

We would like to express our thanks to the members of Kochi Prefectural Fisheries Station and Nansei Mariculture Center in Mie Prefecture for help with the collecting of seawater samples and the measurement of seawater temperature. We also thank Dr. M. Maeda of the Nansei National Fisheries Research Institute for his critical reading of the manuscript.

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IMPACT OF MARICULTURE ON THE SPATIAL AND TEMPORAL PATTERNS OF THE MACROBENTHOS IN GOKASHO BAY

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ABSTRACT

In order to clarify the influence of mariculture on the bottom fauna, samples of the macrobenthos were collected from Gokasho Bay, where fish and pearl farming have been intensively carried out. Five assemblages were identified by cluster analysis of a bay-wide survey conducted in April and August 1993. Four assemblages were associated with the sediment type which changes along the axis of the bay. The other assemblage, which had a distinct pattern of species composition, community structure and seasonal fluctuations, appears to be related to the effect of the fish farms. There was no clear difference in the species composition between the site of pearl farms and the adjacent area where no farming occurred. Monthly samples collected from the fish farm and pearl farm sites during June 1995 to July 1996 revealed that the community structure of the two sites showed distinct differences in seasonal variations. At the fish farm site, near or complete azoic conditions were found from July through November; after December, however, diversity increased markedly through successive recruitments of small-sized species such as the polychaetes *Capitella* sp. and *Pseudopolydora paucibranchiata*, and the amphipods *Aoroides* spp. and *Caprella gigantochir*; the density, biomass and species richness peaked from March to April. At the pearl farm site, there were no clear seasonal trends in the species composition and community parameters. These results show the large impact by fish farming on the macrofauna, while pearl farming causes little or no effect on the benthic fauna. We suggest that the difference in the level of organic input between the two sites results in the differences in the dissolved oxygen content of the bottom water, sulfide content of the sediments, and subsequently the macrobenthic assemblages.

INTRODUCTION

Environmental deterioration around mariculture farms has been conspicuous in many Japanese coastal areas. Several reports have shown that organic wastes discharged from Japanese fish farms cause the deoxygenation of the surrounding waters[1], and changes in the sediment chemistry and macrofauna[2-6]. At pearl farms, deposition of feces and pseudofeces of cultured pearl oysters, and exfoliated fouling organisms also cause environmental deterioration[7,8]. Clear evaluation and minimization of the impacts of farming are necessary from standpoints of both farm management and also for nature conservation.

One of the authors (H. Y.) reported on the effects of fish farming on the macrofauna of Gokasho Bay, and compared the data to other localities suffering from hypoxia at the 24th UJNR Panel Symposium held at Corpus Christi, 1995[9]. In this paper, we will describe the macrofauna of the whole area of Gokasho Bay, and compare the seasonal fluctuations of the macrobenthos between the fish farm and pearl farm sites.

MATERIALS AND METHODS

Study area

Gokasho Bay (34°19′ N, 136°40′ E), which has an area of 22.2 km^2 and a mean depth of 12.7 m, is a typical embayment where mariculture farms are densely distributed. In this bay, fish farming has steadily developed since the introduction of yellowtail culture in 1962. Since 1976, the production of cultured fish including yellowtail and red sea bream in the bay has been over 1500 metric tons. In 1995, fish cages covered 3.6 ha of the bay, where 1760 metric tons of fish were produced. Prior to the

development of fish farms, Gokasho Bay was famous for its high productivity of cultured pearls. In recent years, the production has decreased to a level as low as one eighth of its peak in the 1960s. In 1995, rafts of pearl oysters (*Pinctada fucata martensii*) covered 7.9 ha of the bay producing 800 kg of pearls. The fish farms and the pearl farms are distributed in separate parts of the bay; the fish farms are concentrated in a small inlet, Hazama-ura, while the pearl farms are chiefly distributed at the inner part of the main basin (Figure 1).

Sampling and data analysis

A survey of the macrobenthos was conducted in Gokasho Bay on 20-21 April and 11-12 August 1993. Two replicate samples were collected from 16 stations (Figure 1) by a 0.04 m^2 Ekman-Birge grab and a 1-mm sieve. Sediment samples were also obtained for analysis of particle size, ignition loss (IL), chemical oxygen demand (COD), and the total sulfide content. Dissolved oxygen (DO) of the bottom water (0.5-1.0 m above the bed) was measured using a DO meter (YSI model 58).

A monthly collection of samples was made during the period from June 1995 to July 1996. Three replicate samples were collected from Sta. 5 (depth, 18.4 m; 40 m from the edge of the fish cage) at the center of the fish farm area in Hazama-ura, and Sta. 16 (depth, 14.1 m; 20 m from the edge of the raft) in an area with a high density of pearl culture. A water sample from just above the bed was obtained by a corer, and DO was measured by the Winkler method. The total sulfide content in the sediment (upper 3 cm layer) was determined using a H_2S-absorbent column.

After sorting, the number of individuals per species and, after blot drying with tissue, the weight including shell to the nearest 1 mg was recorded. Kimoto's similarity index $C\pi$ [10] and the cluster analysis[11] were used to identify distinct assemblages in the bay. $C\pi$ was determined from the combination of the April and August 1993 samples. The identified assemblages were tested by the ABC (abundance, biomass comparison) method[12] against samples collected in April 1993 to judge the degree of environmental disturbance. In this method, species are ranked in decreasing order for abundance (number of individuals) and biomass, and the cumulative percentage of each parameter is plotted against the rank. To evaluate the species diversity, the Shannon-Weaver function H', the species richness index H'max and the evenness index J'[13] were adopted.

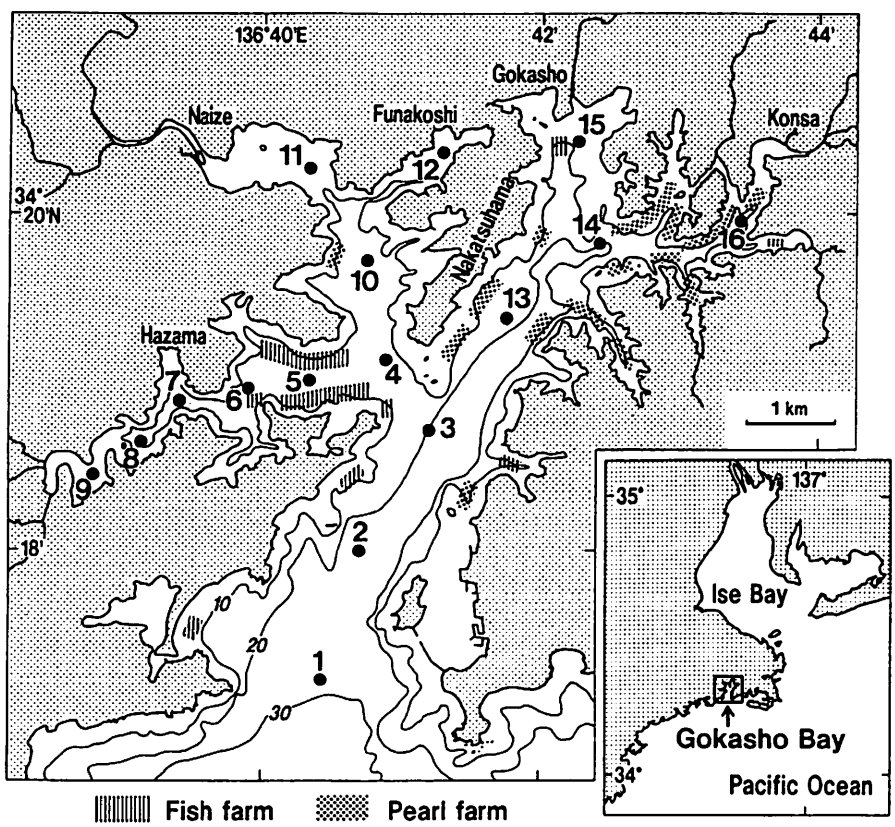

Figure 1. Map of Gokasho Bay showing the sampling stations with depth contours in meters and the areas of fish farms (hatched areas) and pearl farms (stippled areas).

RESULTS AND DISCUSSION

Spatial patterns of macrobenthos and environmental factors

The dendrogram for the 1993 samples was divided into five distinct clusters, lettered A through E (Figure 2). A, B and C are distributed along the main axis of the bay, while D and E are located in Hazama-ura Inlet.

In areas A, B and C community parameters (Table 1) and environmental factors (Table 2) varied gradually between sites. As the location shifts toward the mouth of the bay, the density in August increased, biomass in both months decreased, and the species richness index H'max in both months increased; DO in August increased; silt-clay fraction, COD, and the total sulfide content in the sediment decreased. This spatial tendency suggests that the macrofauna is strongly influenced by the gradient along the main axis of the bay which results in the change in salinity, dissolved oxygen in the bottom water, and the nature of the sediment. Similar conditions have been widely observed in other Japanese embayments, which is known as the arrangement of macrofauna along the axis of a bay[14].

Differences occurred in species composition between the inner part of the main basin "C" and the inner part of Hazama-ura "D" (Table 3) ; the polychaetes *Cossura duplex*, *Spiochaetopterus costarum*, and *Nephtys oligobranchia* were the main constituent species in "C", but these species were scarce in "D" ; the polychaete *Paraprionospio* sp. (form A) dominated in "D", but was rare in "C". The dominance of *Paraprionospio* sp. (form A), an indicator of enriched sediment associated with oxygen deficiency[15], suggests that "D" is more stagnant and eutrophic, compared with "C". The high values of the silt-clay fraction, COD and total sulfide in the sediment, and the low DO concentration in August in "D" (Table 2) support this suggestion.

Species composition and community parameters in "E", where fish farms are densely distributed, have distinct features, in comparison with the other parts of the bay (Tables 1, 3). High density and biomass values occurred in April, while in August, near azoic conditions were

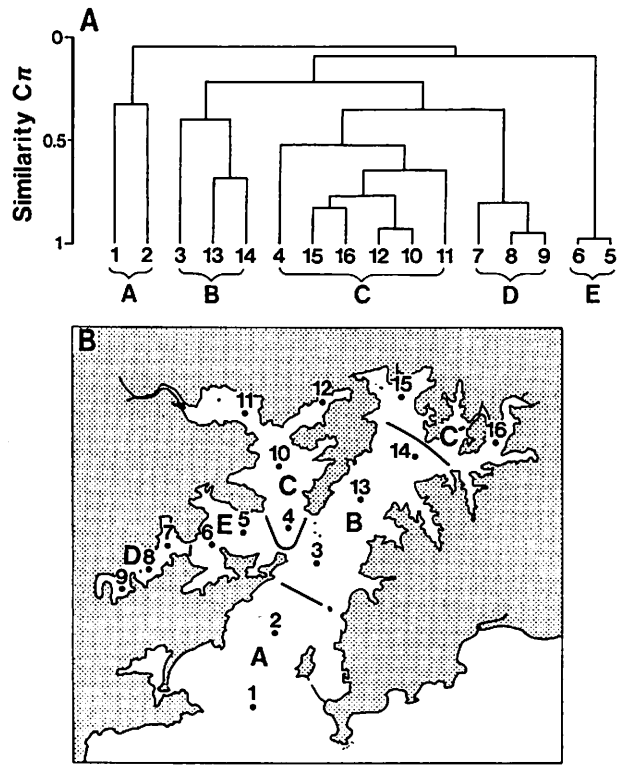

Figure 2. Cluster analysis of the macrobenthos in Gokasho Bay; (A) dendrogram showing similarity between the stations, (B) division of the bay into zones A-E. The Kimoto's index Cπ ranges from 0 (lowest) to 1 (highest).

Table 2. Average values of environmental factors in the five zones of Gokasho Bay.

Zone	Bottom Water*1 Dissolved oxygen mg/L		Sediment Silt-clay fraction %	Median diameter φ	Ignition loss %	COD mg/g dry	Total sulfide mg/g dry
	Apr	Aug	Avg*2	Avg*2	Avg*2	Avg*2	Avg*2
A	6.9	6.0	1.6	2.4	2.9	1.5	ND*3
B	7.2	5.6	59.7	4.8	8.4	10.9	0.14
C	7.3	3.7	75.8	5.4	11.4	12.8	0.68
D	7.0	1.6	84.3	6.7	13.7	18.0	0.72
E	4.8	1.2	58.3	4.8	13.3	16.3	0.58

*1 0-1m above the seabed.
*2 the average of measurements in April and August.
*3 not detected.

Table 1. Average values of community parameters in the five zones of Gokasho Bay.

Zone	Density individuals/m²		Biomass wet weight g/m²		H' bit		H'max		J'	
	Apr	Aug	Apr	Aug	Apr	Aug	Apr	Aug	Apr	Aug
A	3590	2550	11.9 (11.9)	8.7 (8.7)	5.3	4.2	6.3	5.5	0.84	0.77
B	5290	2540	27.1 (27.1)	15.7 (15.7)	5.1	4.6	6.1	5.4	0.84	0.84
C	4450	2120	44.1 (42.0)	18.2 (18.2)	4.3	3.7	5.6	4.9	0.77	0.77
D	5150	1150	57.4 (46.4)	11.3 (11.3)	3.2	1.4	4.8	2.5	0.67	0.61
E	14700	69	57.7 (57.7)	0.1 (0.1)	2.5	0.7	5.7	0.8	0.45	-

( ), excluding animals >1g. −, not computable.

Table 3. Dominant species in the five zones of Gokasho Bay. The three species which ranked highest in number (N, individuals/m^2) or in wet weight (W, g/m^2) are given.

Zone	Species	N	%*	Species	W	%*
April 1993						
A	*Spiophanes bombyx*	594	16.5	unidentified Ascidiacea	3.6	30.1
	Spio filicornis	213	5.9	*Myadropsis brevispinosus*	1.1	9.0
	unidentified Nemertinea	131	3.7	*Spiophanes bombyx*	0.8	6.9
B	unidentified Polycirrinae	321	6.1	unidentified Ascidiacea	4.4	16.1
	Lumbrineris longifolia	263	5.0	*Terebellides kobei*	2.6	9.7
	Chaetozone sp.	238	4.5	*Lyonsia ventricosa*	1.7	6.2
C	*Theora fragilis*	973	21.9	*Theora fragilis*	13.4	30.3
	Lumbrineris longifolia	473	10.6	*Patinapta ooplax*	6.0	13.6
	Cossura duplex	321	7.2	*Paraprionospio* sp. (form A)	4.8	10.9
D	*Prionospio pulchra*	1800	34.9	*Paraprionospio* sp. (form A)	28.6	49.9
	Theora fragilis	683	13.3	unidentified Ascidiacea	11.0	19.2
	Paraprionospio sp. (form A)	571	11.1	*Theora fragilis*	7.2	12.5
E	*Pseudopolydora paucibranchiata*	9210	62.7	*Pseudopolydora paucibranchiata*	12.2	21.1
	Lumbrineris longifolia	950	6.5	*Paraprionospio* sp. (form A)	8.5	14.7
	Prionospio pulchra	619	4.2	*Theora fragilis*	7.4	12.7
August 1993						
A	*Polydora flava orientalis*	838	32.8	unidentified Nemertinea	1.6	18.2
	Aricidea neosuecica nipponica	131	5.1	*Prionospio paradisea*	1.4	16.6
	Spiophanes bombyx	119	4.7	*Polydora flava orientalis*	0.8	9.1
B	unidentified Capitellidae	267	10.5	*Phylo fimbriatus*	3.6	23.1
	Theora fragilis	208	8.2	unidentified Capitellidae	2.6	16.6
	Sigambra tentaculata	179	7.0	*Lumbrineris latreilli*	1.2	7.9
C	*Cossura duplex*	315	14.8	*Phylo fimbriatus*	4.3	23.4
	Theora fragilis	302	14.2	*Terebellides kobei*	3.1	17.1
	Nippopisella nagatai	185	8.7	*Patinapta ooplax*	2.2	12.3
D	*Paraprionospio* sp. (form A)	771	67.3	*Paraprionospio* sp. (form A)	10.6	94.3
	Prionospio pulchra	183	16.0	*Spiochaetopterus costarum*	0.5	4.6
	Spiochaetopterus costarum	88	7.6	*Lumbrineris longifolia*	0.05	0.5
E	*Lumbrineris longifolia*	31	45.5	*Lumbrineris longifolia*	0.05	71.4
	Paraprionospio sp. (form A)	19	27.3	*Paraprionospio* sp. (form A)	0.01	17.9
	Prionospio pulchra	13	18.2	*Prionospio pulchra*	<0.01	8.9

* Percentage of the total density and total biomass of the macrobenthos, respectively.

observed. The polychaete *Pseudopolydora paucibranchiata* overwhelmingly dominated in April, resulting in a low value of the evenness J', and the polychaetes *P. paucibranchiata* and *Capitella* sp., and the amphipod *Protomima imitatrix* were concentrated in "E"[16]. The occurrence of species specific to the fish farm area "E" resulted in a distinct species composition in this area, while the species composition at the pearl farm site (Sta.16) resembled those of the inner part of the bay (Sta. 10, 12, 15) where no farming occurred (Figure 2).

The response of the five assemblages to environmental disturbance was tested by the ABC method (Figure 3). The abundance curve in assemblage "E" lies above the biomass curve, indicating a grossly disturbed condition, while the contrary position of the two curves in other four assemblages indicates little or no disturbance.

These results indicate that fish farming changes the community structure and species composition of the macrobenthos, while pearl farming has no distinct effects on the benthic fauna.

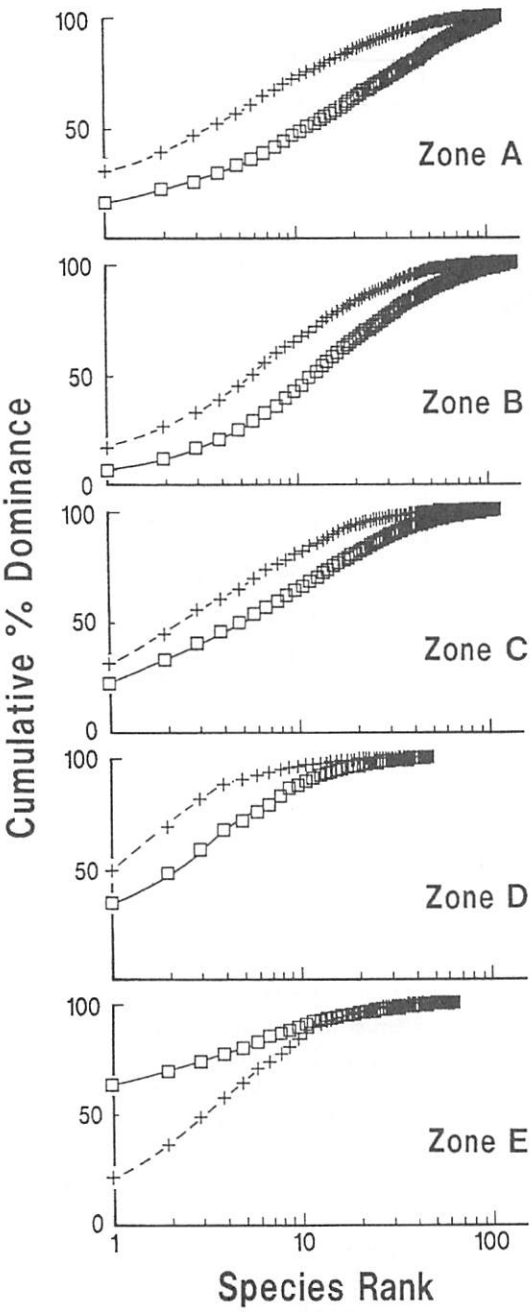

Figure 3. Combined *k*-dominance curves for species biomass (crosses and dashed lines) and number of individuals (squares and continuous lines) in five zones.

Seasonal fluctuations at the fish farm and pearl farm sites

The bay-wide survey showed remarkable changes in the macrofauna between April and August. Therefore, to detail the seasonal fluctuations of the macrobenthos at the fish farm (Sta.5) and pearl farm (Sta.16) sites, investigations were carried out over a 14-month period.

Results show a conspicuous seasonal fluctuation at the

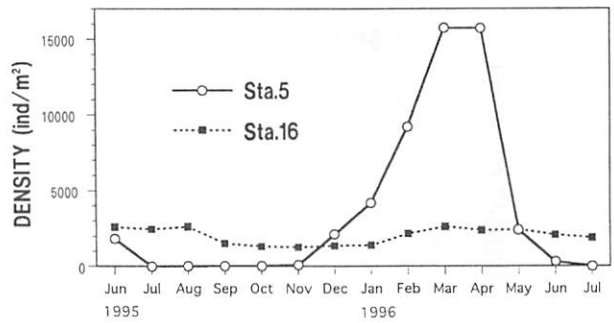

Figure 4. Seasonal fluctuations in the density of the macrobenthos at the fish farm site (Sta. 5) and the pearl farm site (Sta. 16).

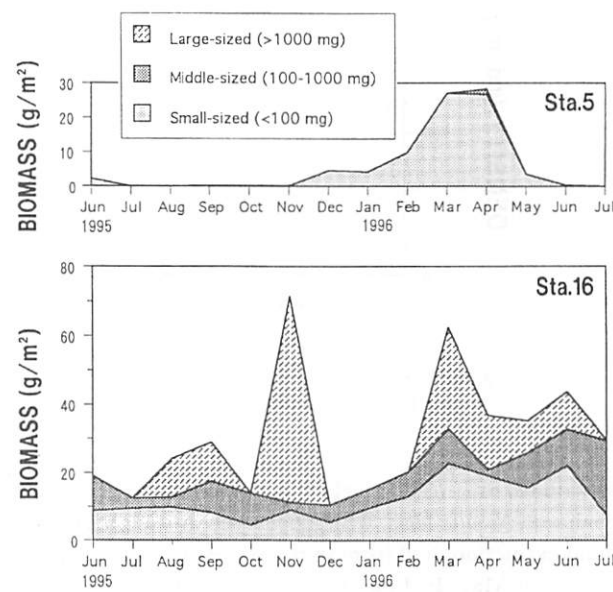

Figure 5. Seasonal fluctuations in the biomass (wet weight) of the macrobenthos at the fish farm site (Sta. 5) and the pearl farm site (Sta. 16).

fish farm site (Figures 4, 5). At this site, near or complete azoic conditions lasted from July through November 1995, and from December, the density, biomass, and number of species increased rapidly attaining a maximum (15700 individuals/m^2, 28.2 g/m^2, 46 species/0.12 m^2) from March to April 1996, before they decreased suddenly in May, and azoic conditions were observed again in July.

The faunal recovery at the fish farm site occurred by successive colonizations of species (Figures 6, 7). The polychaete *Capitella* sp. was the first species which colonized this azoic bottom, and attained a maximum density (6300 individuals/m^2) in February. The polychaete *P. paucibranchiata* was the second to recruit, reaching maximum densities (4100-4300 individuals/m^2) from March to April. The amphipods *Aoroides* spp. and *Caprella gigantochir* had similar recruitment patterns, i.e., population increased after February, and maximum densities (2600 and 3000

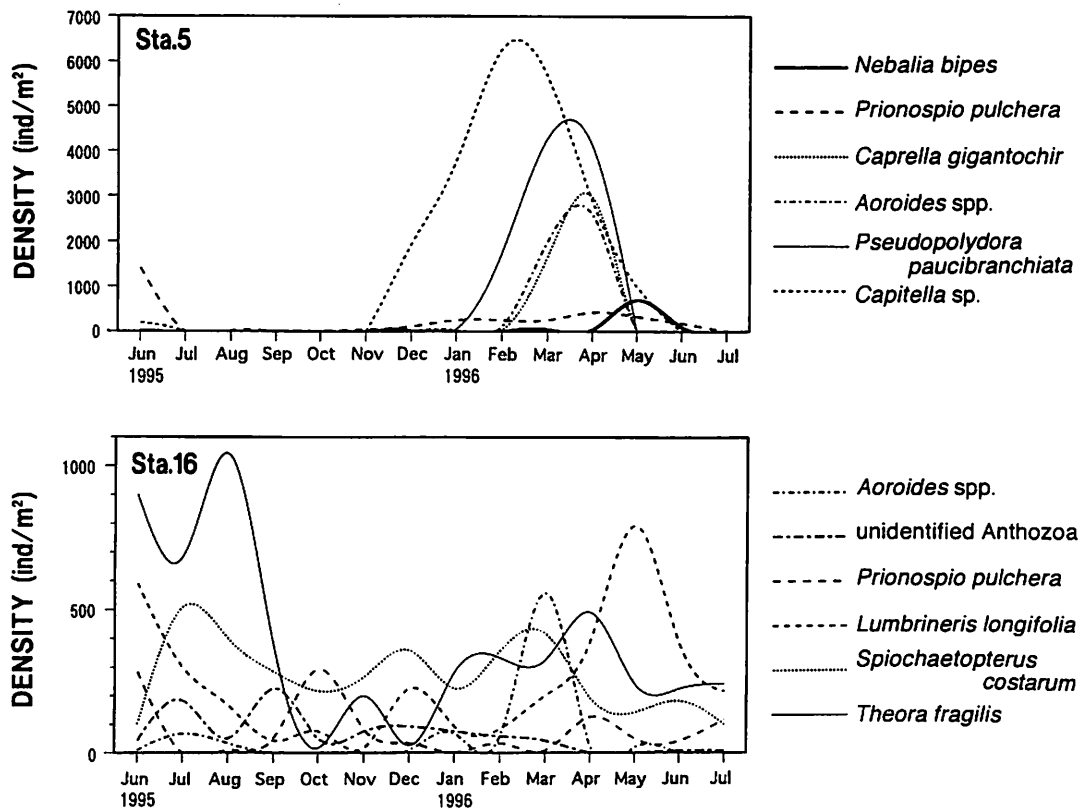

Figure 6. Seasonal fluctuations in the density of the main species at the fish farm site (Sta. 5) and the pearl farm site (Sta. 16).

individuals/m^2, respectively) occurred in April. The nebaliacea *Nebalia bipes*, which occurred at the phase of the faunal extinction, had its maximum density (700 individuals/m^2) in May. In June, the polychaete *Prionospio pulchra* ocurred as one of the last surviving species. Thus, these species excluding *P. pulchra* all had a clear peak in density and the species which colonized earlier had a higher maximum density.

Seasonal fluctuations in the species diversity indices reflected the recovering and subsequent degenerating processes of the macrofauna in the fish farming area (Figure 8). During the earliest stage in the faunal recovery, H' was low due to the occurrence of only a few pioneering species and the dominance of *Capitella* sp. which accounted for $>$ 90 % of the total abundance from December to January (Figure 7). As subsequent species recruited, the species richness H'_{max} increased, and with the dominance by a single species reduced, increases of the evenness J' occurred. Many species disappeared suddenly in May, resulting in decreases of H'_{max} and H'.

The seasonal fluctuations of the macrobenthos at the pearl farm site were in marked contrast to those at the fish farm site. Azoic conditions were not observed at the pearl farm site. Further, there were no clear trends in any of the density (Figure 4), biomass (Figure 5), species diversity indices (Figure 8) and species composition (Figure 7). The main constituent species such as the polychaetes *Spiochaetopterus costarum* and *Lumbrineris longifolia* occurred throughout the year, and showed no distinctive fluctuations in densities (Figure 6). At this site, any overwhelming dominance by a single species did not occur throughout the year (Figure 7), resulting in keeping values of the evenness J' higher than those at the fish farm site (Figure 8).

There were also marked differences in the community structure between the two sites. The assemblage at the pearl farm site consisted of a variety of species of various sizes (Figure 5) ; middle-sized individuals (100-1000 mg) such as the polychaetes *Phylo fimbriatus*, *Terebellides kobei*, *Sthenolepis* sp., *Streblosoma* sp. and *Lagis bocki*, the bivalves *Macoma incongrua*, *Fulvia hungerfordi* and *Pillucina pisidium*, the holothurian *Patinapta ooplax*, and an unidentified Ascidiacea occupied 26.1% of the total weight; large-sized individuals ($>$ 1000 mg) such as the polychaete *Phylo fimbriatus*, the bivalves *Macoma incongrua* and *Paphia undulata*, and an unidentified Ascidiacea occupied 35.0%. By contrast, all specimens collected from the fish farm site except a juvenile sea eel *Conger myriaster* (177 mg) belonged to the category of small-sized animals ($<$ 100 mg). Thus, the community structure at the pearl farm site was

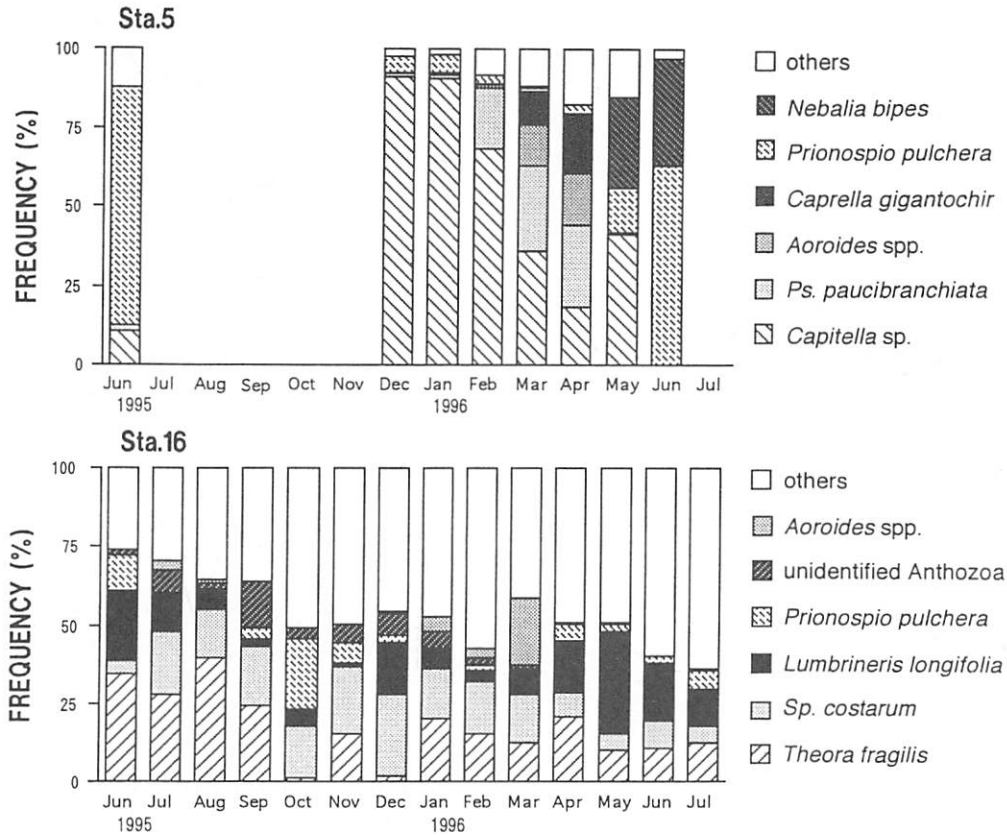

Figure 7. Seasonal fluctuations in the numerical composition of the macrobenthos at the fish farm site (Sta. 5) and the pearl farm site (Sta. 16).

more diverse and stable throughout the year, as compared with that at the fish farm site.

Impact of farming on the macrofauna

The present survey showed azoic conditions at the fish farm site during the summer and autumn (Figure 4). It has been shown that a low oxygen level is a key factor in eliminating the macrofauna in organic-enriched habitat[17]. However, it is difficult to explain this from the observed DO values which were >2.8mg/L during the sampling period in 1995 (Figure 9). Abo *et al.*[18], and Abo and Toda[19] observed, however, that the oxygen concentration of the bottom water in Hazama-ura was subject to repetitive fluctuations ranging from 5 mg/L to anoxic, within a period of 10-15 days during the summer. It is probable that low and lethal, but unrecorded concentrations of oxygen caused the defaunation at this site.

Another conspicuous occurrence at the fish farm site was the rapid increase in density after December (Figure 4). Monthly observations of Gokasho Bay during a period of 1989-1996 indicate that the oxygen content of the bottom water at the fish farm site does not fall below 3 mg/L after October. This finding suggests that two months after the recovery of the oxygen content are required for successful recruitment. There may be other factors which also inhibit the recruitment.

Total sulfide content in the sediment of the fish farm site was over 1.3 mg/g dry sediment from August through October and in December (Figure 10). Thereafter, as the density of the macrobenthos increased, the sulfide content decreased until April, when a minimum sulfide value (0.63 mg/g) and a maximum density value (15700 individuals/m^2) were observed. Thus, there is a significant negative correlation between the sulfide content and the density of the macrobenthos after the beginning of the faunal recovery in December ($p<0.01, r=-0.866$) (Figure 11). This finding suggests that the hydrogen sulfide content in the reduced sediment inhibits the initial recruitment of benthic animals, but that recruited animals oxidize the sediment by their bioturbation and irrigation activities, resulting in a decrease in the sulfide content of the sediment.

At the fish farm site, a faunal recovery was attained by successive recruitments of species in order of *Capitella* sp., *Pseudopolydora paucibranchiata*, and the amphipod species. The *Capitella* species complex (especially, species I) is regarded as a typical opportunistic species, a short-lived species adapted to unpredictable environments by

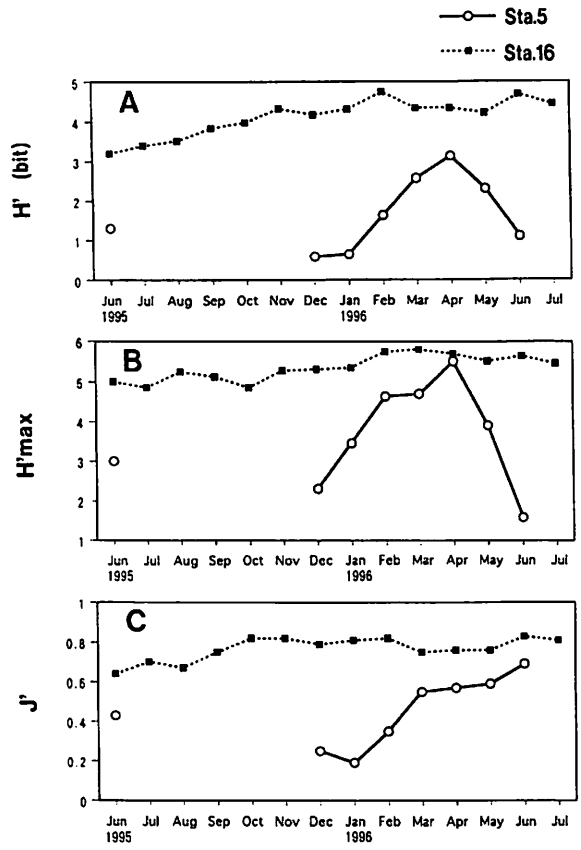

Figure 8. Seasonal fluctuations in the species diversity indices at the fish farm site (Sta. 5) and the pearl farm site (Sta. 16) ; (A) the Shannon-Weaver's diversity index H', (B) the species richness index H'max, (C) the evenness index J'.

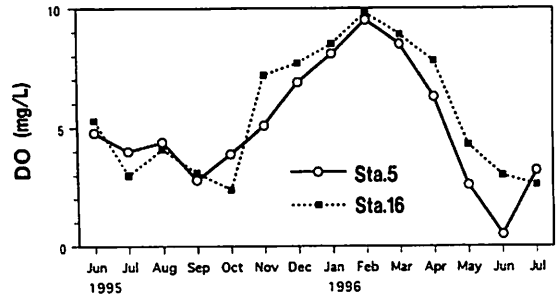

Figure 9. Seasonal fluctuations in the dissolved oxygen content of the bottom water just above the seabed at the fish farm site (Sta. 5) and the pearl farm site (Sta. 16).

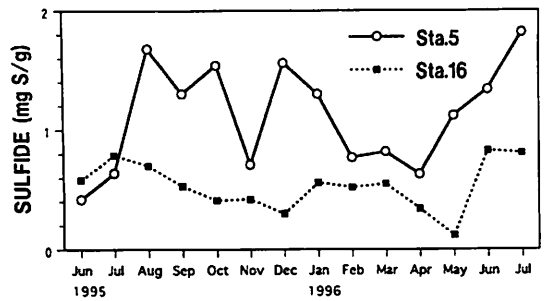

Figure 10. Seasonal fluctuations in the total sulfide content in the sediment at the fish farm site (Sta. 5) and the pearl farm site (Sta. 16).

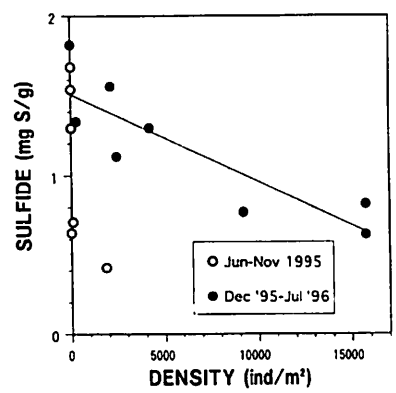

Figure 11. Relationship between the density of the macrobenthos and the total sulfide content in the sediment at the fish farm site (Sta. 5). Open circles indicate values obtained during the period of June to November 1995, and closed circles indicate December 1995 to July 1996.

virtue of its ability to reproduce rapidly[20]. *Capitella* species has often been reported from fish farm areas in Europe[21], and in Japan[2-6]. It has been shown that nutrient enrichment is positively related to individual growth, population growth, and fecundity of *Capitella* species[22-26]. It has also been suggested that micronutrients such as amino acids and fatty acids may limit the growth and reproduction in *Capitella* sp. I[27,28]. Deposition of organic wastes from fish cages into the sediment appears to provide an enhanced food supply to *Capitella* sp., which feeds nonselectively on subsurface sediments containing decayed organic material and associated microbes[29]. The density of this species decreased drastically before the beginning of the environmental deterioration in May. The observed decline in the *Capitella* population after February may be caused by a food shortage, if organic rich sediment containing available food is indispensable for maintaining a large population of *Capitella* sp., and if this food supply has already been consumed during high density phase of this species.

P. paucibranchiata, the second species to re-populate at the fish farm site, feeds selectively on organic-mineral aggregates and suspended organic particles[29,30], which are unavailable directly to the *Capitella* species. The population growth of *P. paucibranchiata*, however, needs to be supported by a steady input of nutrient-rich particles in the form of unconsumed food particles and fish feces which accumulated on the sediment-water interface.

As the faunal recovery proceeds at the fish farm site, *Aoroides* spp. and *Caprella gigantochir* increased their

densities, together with increases in species richness and evenness of the macrofauna. The most diverse assemblage was found in April. At this time, several individuals of the *Aoroides* species were found in the stomach of the sea eel *Conger myriaster* sampled at this site. This observation indicates the probable food chain in the benthic community at the fish farm site. However, this assemblage tended toward extinction abruptly in May, probably due to the deoxygenation of the bottom water accompanied by increasing temperatures and increasing activities of fish farming.

Gown *et al.*[31] pointed out that a reduction in species richness, an increase in the total abundance instead of a decrease in echinoderms, and high composition of small-sized organisms are common features of the macrobenthos in the vicinity of mariculture facilities. Similar phenomena were also observed in fish farm areas in Tomoe Cove[2,4-6], and in Gokasho Bay. Comparison of the species composition in 1993 with a previous survey conducted in 1941 by Miyazi and Masui[32] indicates that the spatangina *Schizaster lacunosus* has disappeared from the fish farm area in this bay[16]. The ABC method, which shown as a sensitive indicator of disturbance caused by fish farming[33], also showed the fish farm site in Gokasho Bay to be grossly disturbed (Figure 3). This result is principally due to the lack of large-sized species and the numerical dominance of small-sized species, i.e., *P. paucibranchiata* which contributed a relatively small proportion of the biomass.

Gown and Bradbury[34] described a gradient of enrichment away from fish cages; an area directly beneath fish cages is azoic and in the immediate vicinity around the farm, the macrofauna is impoverished and dominated by opportunistic species. In the present study area, this spatial pattern appeared to seasonal fluctuate at the fixed station.

All of these features observed in the present survey suggest that organic wastes from fish farms affect the macrobenthos in a similar manner to those typically observed along gradients of other effluents of domestic or industrial organic wastes as described by Pearson and Rosenberg[17]. On the other hand, the assemblage at the pearl farm site was in marked contrast to the assemblage at the fish farm site; a higher diversity including large-sized species occurred, species composition was similar to those in adjacent areas with no facilities for pearl farming, and no obvious seasonal fluctuation in the community parameters or species composition occurred. These findings and the analysis by the ABC method indicate an undisturbed condition occur at the pearl farm site. At the pearl farm site, hypoxic waters below 2.4 mg /L of DO were not observed at any time in the present survey (Figure 9). Anoxic conditions (DO, $<$1.0 mg/L) also were not found during the monthly observations conducted in 1989-1996. The total sulfide content at this site was low (0.12-0.83 mg /g), compared to the content at the fish farm site (0.63-1.8 mg/g) (Figure 10). These results suggest that organic enrichment at the pearl farm site is not as conspicuous as it is at the fish farm site.

The amount of discharged particles from a raft of pearl farming including the feces and pseudofeces of cultured pearl oysters, feces of fouling organisms, and exfoliated fouling organisms is estimated to be 17.5 kg/m^2/yr (dry weight)[35]. At the fish farm site, supposing that 30% of feed is discharged into the water in the form of leftovers and fish feces[31,36], the input of particulate organic material into the water of Hazama-ura in 1995 is estimated to be 93 kg/m^2/yr (dry weight). It is concluded that such a difference in the level of organic material input between the two sites results in the contrasting macrobenthic assemblages.

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THE FUNCTIONS OF PRODUCTION AND PURIFICATION BY DIVERSE ORGANISMS ON AN INTERTIDAL FLAT

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ABSTRACT

An investigation on the budget of nitrogen and phosphorus in an intertidal flat has shown that the flat had the purification function of both chemicals because these were diminished by about half of the input from land. An investigation on the various biomass and their metabolic rate of nitrogen and the diminishied nitrogen can be explained by the accumulation into macrophyte (eelgrass and sealettuce) uptake on land by the fishery and the denitrification. Moreover, the fish in the intertidal flat feed on various food because of the existence of different organisims. The flat had a large purification function and high production function by the cooperation of the diverse organisims in the flat. Therfore, protection of intertidal flats from development such as reclamation to sustain biodiversity of organisms in these habitats is crucial to keep fishery fruitful.

INTRODUCTION

Intertidal and surrounding shallow zones are very important for human life because these areas are thought to be highly productive in fisheries, and serve as nursery grounds for many species of juvenile fish, human recreation, purification of pollutants and resting place for migratory birds. The same zones have been the areas for extensive human activities such as industry and harbor development, and wide reclamations have been carried out on coastal areas of Japan since 1950. Since then, the movement against the reclamations has spread in many areas in Japan since the intertidal flats and shallow areas have been become scarece. People demand that researchers clarify the reason why a shallow area, especially an intertidal flat is important in ecology and fisheries. We, therefore, investigated the function of production and purification of Issiki intertidal flat from 1982 to 1986 supported by Environment Agency. In this paper, the diverse organisms which play a significant role in the purification and the biological production in the flat are reported.

METHOD

The functions of purification and biological production were investigated by the analysis of budgets and circulations of nitrogen and phosphorus on the flat. Nitrogen circulation was investigated by two methods. One is the estimation from the biomass and the metabolic rates of the flat ecosystem components and another is by box model analysis.

Description of the Issiki intertidal flat

The Issiki tidal flat discussed here is located in Mikawa Bay, a large eutrophicated bay in the middle part of Japan facing to Pacific Ocean. The flat is a typical foreshore flat of about 10km in length and about 1.5km in mean width. The flat has a terrace shape inclining very gently to its margin of 2 to 3m in depth and therefrom very steeply to the estuarine bed of 5 to 6m in depth as shown in Figure 1 Dimensions of the flat are shown in Table 1. In the spring tide, a large part of the flat is exposed to air at low water, while most of it submerges to a depth of 2 to 3m at high water. Fresh water and nutrients are supplied through some small rivers and watergates. The nutrient supply originates from fertilizers on the farmland, the domestic sewage and the eel culture sewage in the background area. These nutrients support the growth of macrophytes (*Ulva pertus*, *Zostera marina*, etc.) and benthic animals on the flat. The fishery in the flat takes up laver at winter,the short-necked clam mainly from spring to fall, the sealettuce mainly in summer and various fish and shellfish at various seasons. The water temperature changes seasonally from 5 to 30℃. The salinity varies spatially from less than 10 (at the rivermouth) to 30ppt (at the flat margin) in summer, the dissolved inorganic nitrogen from 5 to 10 μM, and the

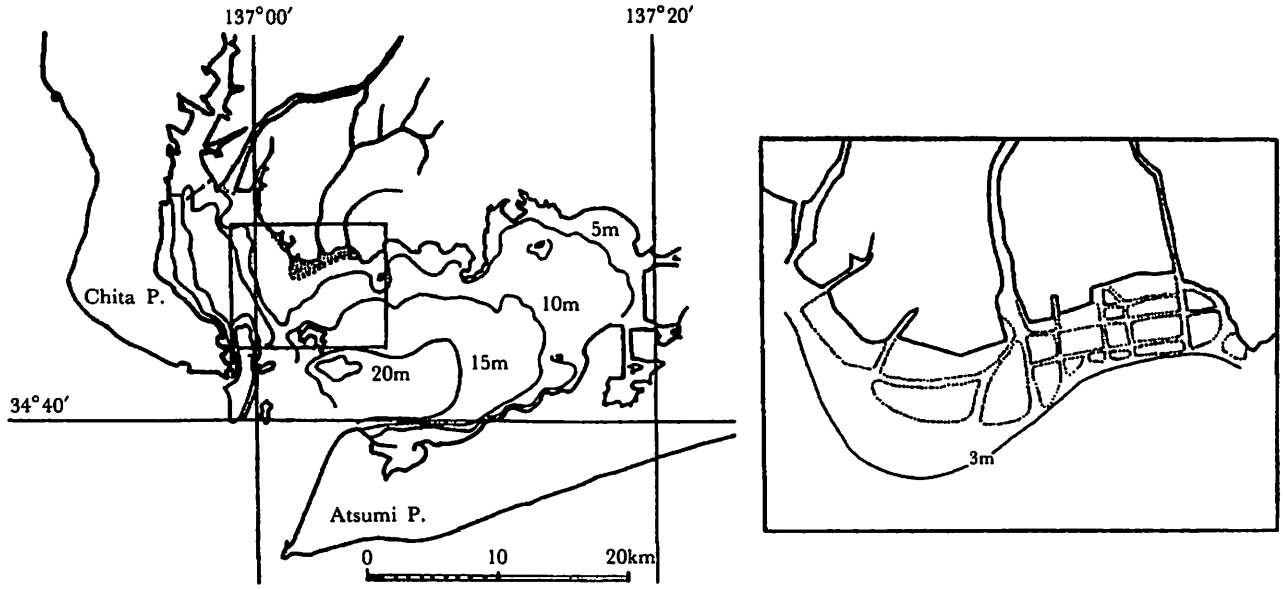

Figure 1. Location of Mikawa Bay (left) and Issiki intertidal flat (right)

Table 1. The dimension of the Issiki intertidal flat

Mean width	1.5km	Mean depth	1.5m
Marginal depth	3.0m	Marginal length	10km
Flat area	9km^2	Water volume	about 14×10^6m^3

dissolved inorganic phosphorus from 1 to 3 μM.

Boxmodel analysis

The metabolic rate such as production and decomposition was estimated as follows[1].

(1) Water budget $\triangle V/\triangle t+AU=Q$, where $\triangle V$ is volume change of water on the flat during observation, $\triangle t$ the observation time, A the crossectional area of margin of the flat, U the velocity at the margin, so AU shows the water amount which flows out of the flat, and Q is the fresh water supply. We estimate U from $\triangle V/\triangle t$, A and Q obtained by observation.

(2) Salt budget $\triangle (VS^*)/\triangle t+A\{USa-Kx\triangle S/\triangle x\}=0$ where S^* is volume-average salt concentration, thus VS^* given the entire salt contents on the flat and $\triangle (VS^*)$ is the change of salt amount during the observation. The term Sa denotes the salt concentration at margin, so USa indicates the salt flow out of the flat, Kx the horizontal dispersion constant, $\triangle S/\triangle x$ the horizontal salt gradient, and thus $Kx\triangle S/\triangle x$ indicates the exchange salt amout between the flat and offshore. We estimate Kx from the another data, too.

(3) Nitrogen budget $\triangle (VN^*)/\triangle t+A\{UNa-Kx\triangle N/\triangle x\}=Pn+Qn$

where N^* is volume-averaged nitrogen concentration, thus VN^* shows all nitrogen amount on the flat and $\triangle (VN^*)$ is the change of nitrogen amount during the observation. The term Na is the nitrogen concentration at margin, Kx and $\triangle N/\triangle x$ are the same in salt budget. The term Qn is the nitrogen load from the background area into the flat. The term Pn was calculated from these data obtained by observation and it implies the changes occurred in the flat which are physical, chemical and biological. For example, when N is dissolved inorganc nitrogen (DIN) and Pn is minus, then it is understood that DIN is utilized by phytoplankton or macrophyte and could estimate the photosynthesis by plants from the values of Pn.

Observations

We observed the distribution of material on the tidal flat and the input of nitrogen and phosphorus in the stations as shown in Figure 2 from 18 to 29, twice (high water and low water) a day, two days apart, July 1984. Open circles showed the obsevation stations on the tidal flat and solid circles were the stations occupied during the input survey.

On the tidal flat, we observed salinity, DIN, DIP, PON, POP and chlorophyll. For the load survey, we observed the water quality such as DIN at the river and watergates. Fresh water supplies from the rivers and the main watergates were estimated from given data of river flow, service waters for city, agricultuture and eel culture, precipitation, evaporation and pump operation at watergates. Nutrients supplies were calculated by means of multiplying the discharge rate of the rivers and

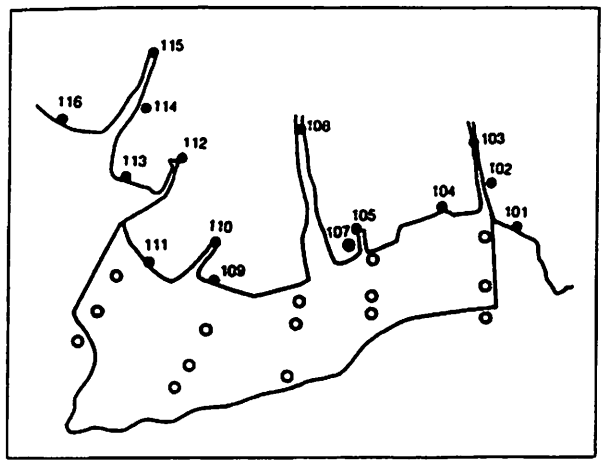

Figure 2. Sample stations on the flat (○) and for the input observation (●)

watergates by the nutrient concentrations observed.

RESULTS AND DISCUSSION

Estimation of biomass and metabolic rate of the ecosystem components

Macrophytes (MP)

Vegetating density of the *Zostera* and *Ulva* on the flat was observed photographically by plane and with the naked eye by boat. The *Zostera* biomass estimated was 30 ton N at the maximum in May and 10 ton N in summer. Its production deduced from a measurement of individual growth rate[2,3] was 0.4 ton N/day. The *Ulva* biomass estimated[4,5] was 40 on N at the maximum in September and 5 ton N in summer and its production estimated from the biomass growth rate, 7% per day, was also 0.4 ton N/day.

Phytoplankton (PP)

The mean chlorophyll-a (chla) concentration in the water column observed was 15 mg/m^2 and taking its N/chla ratio of 5, the PP biomass was calculated to be 0.8 t on N. Further, taking the assimilation number to be 3 mg C/mg chla/hr and solar radiation time to be 12hr and C/N ratio of PP to be 5, the PP production was estimated to be 1 ton N/day.

Benthic Microalgae (BM)

The mean chla density on the flat bed observed was 45 mg/m^2. Taking the same C/chla ratio, the same solar radiation time, the same C/N ratio and a half of the same assimilation number as PP, the biomass of BM was 1.8 ton N and its production 1.5tonN/day.

Bacteria in the water column (BW)

The mean density observed was 5.5×10^6 cells/ml and its growth rate estimated 6.3×10^6 cells/ml/day. Adopting the nitrogen content per cell measured in other flat[6], 0.22×10^{-11}mgN/cell, the biomass was 0.2 ton N and its production 0.2 ton N/day. The NH_4 excretion was taken same to the production.

Bacteria in the bed (BB)

The density observed was 5×10^8cells/g (wet). Taking the specific weight of the bed at 1 g/cm^3 and the habitation depth 5 cm, and taking the N content and the growth rate the same as those in the water column, the biomass was calculated to be 1.0 ton N and the production 1.0 ton N/day. The excretion was also taken the same to the production.

Meiobenthos (MEB)

The *polichaeta* was dominant. The density observed was 2×10^6inds./m^2 or 0.4mgC/m^2 and taking the turnover taime in summer to be twice of the annual mean one, 2 ×9 times/yr, the biomass was 0.8 ton N and the production 0.04 ton N/day.

Macrobenthos (MAB)

The density observed, in which short-necked clam was dominant, was 48g (dry) /m^2 and its N content was 10%. Thus the biomass was 48 ton N. Based on the laboratory experiment, the food intake was estimated to be 1.5 ton N/day, the production 0.2 ton N/day, the pellet excretion 0.6 ton N/day and NH_4 excretion 0.6 ton N/day.

Zooplankton (ZP)

The density in the water column observed was 200mg (dry) /m^2. Taking its N content at 10%, the biomass was calculated to be 0.2 ton N. Based on the size and weight spectrum of ZP observed and the Ikeda-Motoda's empirical relation[7], the food taken by ZP was estimated at 0.2 ton N/day, its production 0.06 ton N/day and the NH_4 excretion 0.1 ton N/day. The biomass and production in the flat described here are shown in Table 2.

Estimation of benthic flux

Assuming complete tidal exchange between the overlying water and the upper interstitial water twice a day, the benthic flux (F) was calculated by the relation, $F=2$ (Ci-Co) Hv, where Ci and Co indicate nutrient salt concentrations of the interstitial and overlying water, H the thickness of tidal exchanging layer and v its porosity[8]. The benthic flux of N salt estimated was 35 mg N/m^2/day, or 0.35 ton N/day.

Table 2. The biomass and production by the organisms in the flat

		Biomass (mgN/m^2)	Production ($mgN/m^2/day$)
Water	Phytoplankton	75	108
	Zooplankton	25	6
	Bacteria	19	22
Macrophyte	Eelgrass	1100	44
	Seakettuce	580	41
Bed	Benthic Algae	183	162
	Bacteria	96	110
	Macrobenthos	4800	22
	Meiobenthos	76	2

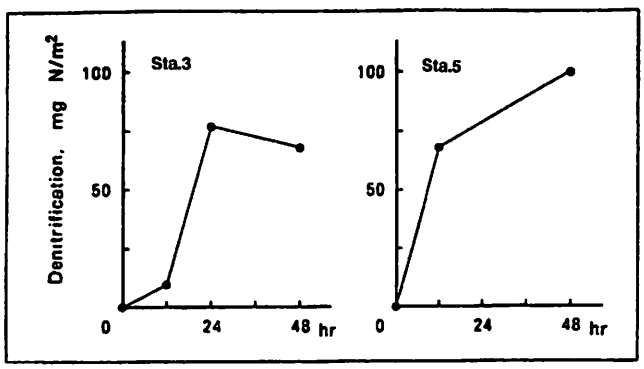

Figure 3. Time course of denitrofication of the flat bed

Estimation of denitrification

Denitrification of the flat bed was directly analysed by gasmetry using the core-sample from the bed. Results were shown in Figure 3. These data were in the range of 50 to 90 mg $N/m^2/day$, or 0.5 to 0.9 ton N/day.

Estimation of uptake on land by fisheries

In the flat, laver was obtained in winter, short-necked clam harvest in spring and Ulva harvest in summer as shown in Figure 4. In summer, N transported on land by fisheries was estimatied to be about 0.19 ton N/day.

Estimation of burying

The vertical profile of ^{210}Pb in the bed was investigated and the profile indicated no burying, so N as well as P was not buried in the flat. The nitrogen fluxes described here were shown in Table 3.

Table 3. Nitrogen flux (tonN/day) in the flat

Macrophyte growth	0.78	eelgrass growth : 0.38 sealettuce growth : 0.4
Nutrients exchange in the bed	0.35	
Denitrification	0.5−0.9	
Uptake by fisheries	0.19	short -neckd clam sealettuce
Burying	nearly 0	^{210}Pb method

Budgets of nitrogen in Issiki intertidal flat

Supplies of freshwater and nutrients

In observation period, freshwater supply into the flat was calculated to be 4.73 m^3/sec. The input of nitrogen was 2.4 ton N/day.

Budget of nitrogen and phosphorus

Table 4 showed the budget of nitrogen in the flat. The term H→L in the most left indicated the budget from high water to low water. The term $\triangle$ (VN*) /$\triangle$t showes the change of stock and. In the case of 7/20, H→L, DIN was much decreased ($-65.6\times10^5\,\mu$ MN/sec) and PON was increased ($20.69\times10^5\,\mu$ MN/sec), finally total nitrogen was decreased ($-44.91\times10^5\,\mu$ MN/sec). The data of other days could be understood the same way. Table 5 showed the budget of phosphorus. Figure 5 showes the Table 4 and 5 pictorially. White bars indicated the budget of DIN, stripe bar PON and black bars total nitrogen. Figure 5 showes that the average budget from 7/20 to 7/26 being minus in all case (DIN, PON and total N), namely nitrogen was diminished from the flat. The average circulation of nitrogen (ton N/day) was showed in Figure 6. This figure indicated that 2.04 of DIN and 0.31 of PON were put into the flat, 1.03 flowed out and the remaining 1.32 was diminished on the flat. This figure implies that about one half of nitrogen input was decreased on the flat, so the flat had a purification function for nitrogen. We examined the trem of 1.32 ton N/day and estimated as described in Table 3, namely, *Ulva* growth (0.40), *Zostera* growth (0.38), fisheries uptake (0.19) and denitrification (0.35).

The circulation of nitrogen in the flat and the purification function

We considerd the quantitative role of organisms on the circulation of nitrogen in the flat and drew Figure 7. About one half of the nitrogen input flowed out of the flat into the bay and the remaining was absorbed by phtoplankton, benthic phytoplankton and macrophytes. The nitrogen absorbed into phytoplankton was grazed by

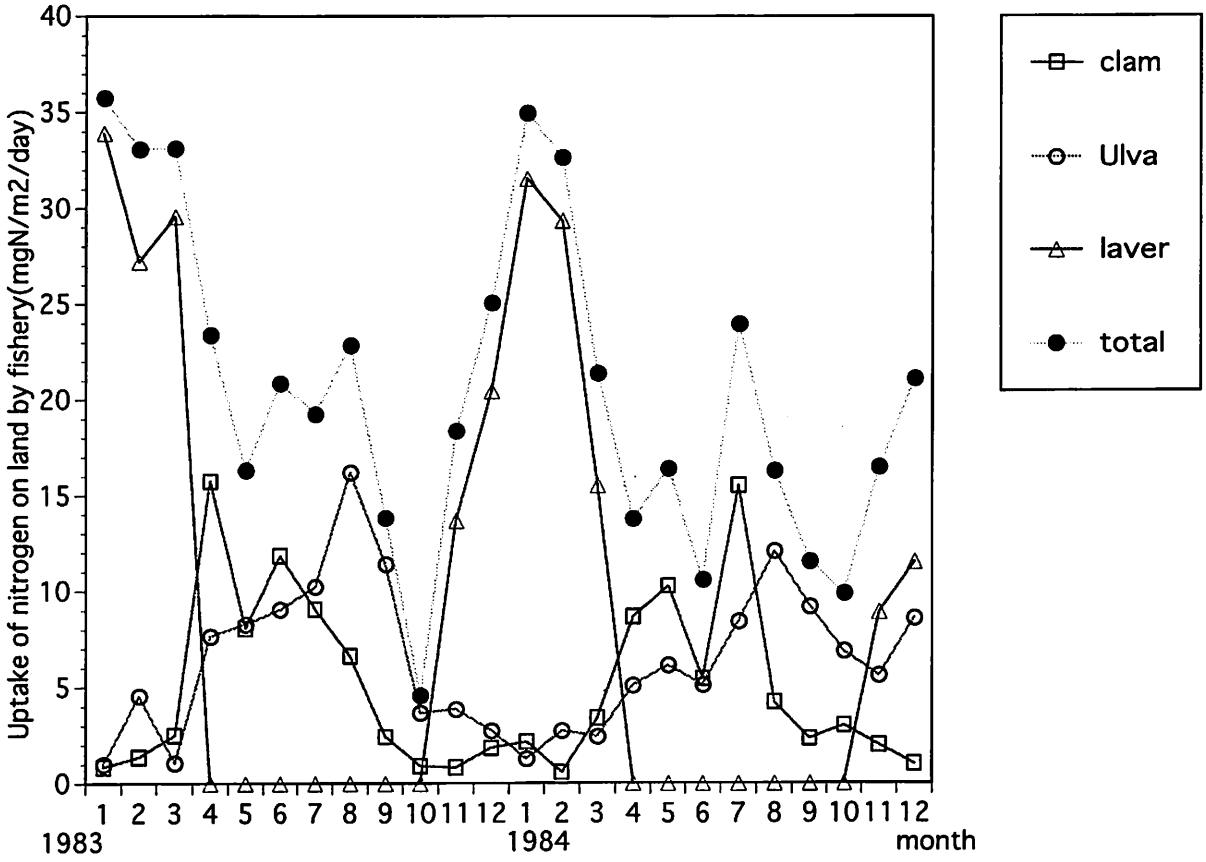

Figure 4. Uptake of nitrogen on land by fishery

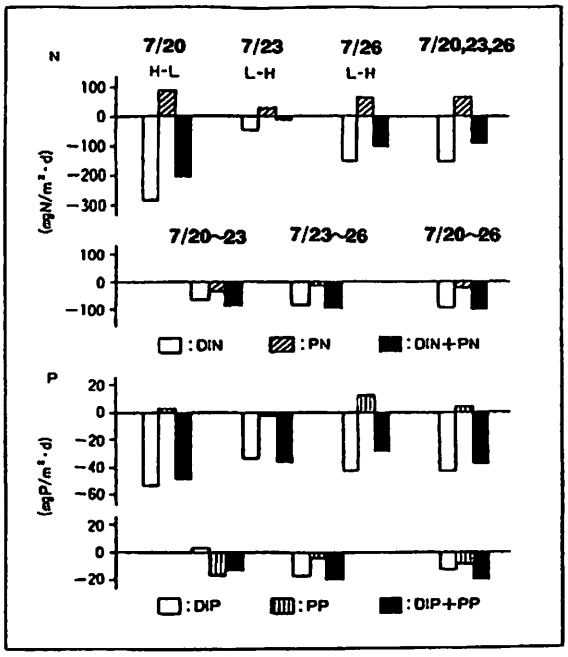

Figure 5. The budgets of nitrogen and phosphorus in the flat during tide-tide and in several days

macrobenthos such as short-necked clam and the nitrogen was transported into the bed as fecal pellte, then denitrificated to nitrogen gas by bacteria. The nitrogen absorbed into macrophytes was used to grow and this nitrogen was accumulated in the flat. Another part of nitrogen was transported on land by fisheries such as *Ulva* and short-necked clam. Generally, one part of nitrogen was absorbed by phytoplankton, circulated by clam, uptaked by fishry and denitrificated and another part of nitrogen was absorbed by macrophytes, accumulated and uptaked by fishry.

Purification function

The Issiki intertidal flat has the ability to decrease the nitorgen and phosphorus inputs from land. The accumulation of nitrogen into macrophytes such as *Ulva* and *Zostera* is temporary because it flows out to the offshore after autumn. However, it is withered and decomposed in autumn or thereafter when thermal convection has already begun and the condition for the oxygen depletion which killed marine organisms has been lost. Therefore, this temporary accumulation of the macrophytes in the flat

Table 4. The budget of nitrogen ($x10^5 \mu$MN/sec)

		$\frac{\Delta}{\Delta t}(VN^*)$	AUN	$-AK\frac{\Delta N}{\Delta x}$	QN	PN	PDN+PPN
7/20	DIN	−48.30	11.30	1.6	30.20	−65.60	
H→L	PON	−0.41	24.8	−0.1	3.6	20.69	−44.91
7/23	DIN	8.13	−8.96	2.93	12.5	−10.4	
H→L	PON	29.37	−18.45	−0.25	2.4	8.27	−2.13
7/26	DIN	−9.02	−20.25	1.21	7.82	−35.88	
H→L	PON	43.54	−29.78	0.18	1.73	12.21	−23.67
7/20	DIN	0.61	0.66	14.03	21.35	−6.05	
→7/23	PON	−1.08	1.41	−0.98	3.00	−3.65	−9.70
7/23	DIN	−1.16	−0.08	2.01	10.16	−9.39	
→7/26	PON	0.40	−0.14	−0.04	2.07	−0.85	−11.24
7/20	DIN	−0.33	0.25	6.08	16.84	−10.84	
→7/26	PON	−0.29	0.49	−0.12	2.58	−2.50	−13.34

Table 5. The budget of phosphorus ($x10^5 \mu$MP/sec)

		(VN^*)	AUN	−AK	QN	PN	PDN+PPN
7/20	DIN	−5.50	2.14	0.25	2.30	−5.44	
H→L	PON	−0.65	2.24	0.03	1.28	0.34	−5.10
7/23	DIN	−1.29	−1.43	0.35	1.10	−3.47	
H→L	PON	1.46	−1.15	0.01	0.52	−0.20	−3.67
7/26	DIN	−0.54	−3.14	0.14	0.71	−4.25	
H→L	PON	5.07	−3.51	0.05	0.25	1.36	−2.89
7/20	DIN	−0.14	0.12	1.89	1.72	0.15	
→7/23	PON	−0.17	0.11	0.12	0.90	−0.84	−0.69
7/23	DIN	−0.18	−0.01	0.24	0.91	−0.86	
→7/26	PON	0.20	−0.01	0.03	0.39	−0.17	−1.03
7/20	DIN	−0.15	0.04	0.87	1.38	−0.62	
→7/26	PON	0.09	0.04	0.09	0.68	−0.46	−1.08

significantly prevents the injurious oxgen depletion int the bay in summer. As above described, the purification function was realized by the cooperation of the diverse organisms in the flat which have fast turnover rate such as phytoplankton, zooplankton, bacteria and slow turnover rate such as macrophyte, macrobenthos. We calculate the purification function to be about 150 mg N/m^2/day and 30 mg P/m^2/day in the flat at summer.

Production function

Nixon[9] demonstrated fisheries yield (FY) per unit area as a function of primary production (PP) per unit area in a variety of esturine and marine system and found the regression line for the marine systems is ln FY = 1.55 ln PP-4.99. In his figure, the highest production is found in esturine area including an intertidal flat and he described that the high production was caused in part by the supply of energy such as tidal one. In addition to the energy supply, I think the factors of the high productivity in estury and tidal flat as follws. 1) supply of nutrients from land by river, 2) supply of oxygen by turbulence of sind and tide in shallow area, 3) various interactions among diverse organisims.

Table 2 shows the biomas and production of various organisims in Issiki tidal flat at summer. The largest biomas was macrobenthos, the second large biomas was macrophyte such as eelgrass and sealettuce. The highest

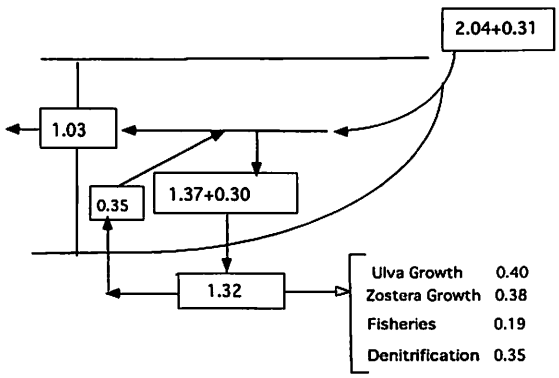

Figure 6. The circulation of nitrogen (tonN/day) in the flat at summer

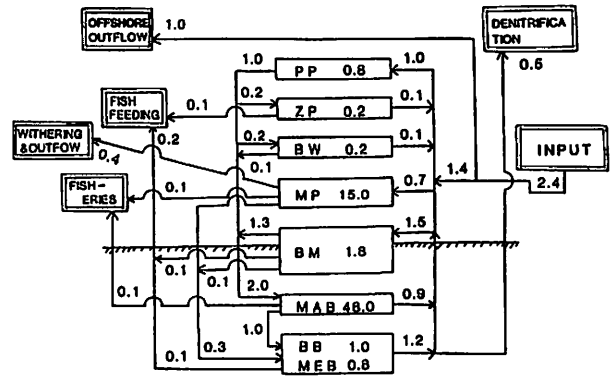

Figure 7. The nitrogen circulation (tonN/day) by ecosystem components of the flat. Abbreviations of the components are described in the text

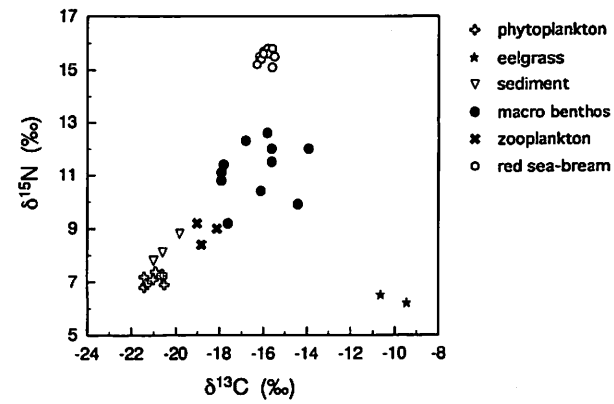

Figure 8. The isotopic δ ratio of stable carbon and nitrogen of various organisms in Shishiki Bay

producer is benthic algae, the second is bacteria in sediment. These results indicate that the ecosystem of tidal flat is made of benthic mainly. But, since macrobenthos such as bivalves get phytoplankton in water as food, the system in sediment and that in water are combined closely as shown in Figure 7 As described in the paragraph of purification, faster and slower primary producers exist in the tidal flat and both primary producer are thought to work for the purification function. In the case of production, the slower primary producer such as eelgrass could be used by secondary producer.

Figure 8 showed the isotopic ratios of stable carbon and nitrogen of various organisims in Shishiki Bay in Nagasaki Prefecture in Kyushu. In general, the isotopic ratio of carbon indicates the carbon source of the target organism and that of nitrogen dose the trophic level of the organism. The nitrogen isotopic ratio of a juvenile red seabream is about 16 and highest, so it is thought to be higherst predator in the bay. Since the N isotpoic ratio of primary producer such as phytoplankton and eelgrass are 7, the difference between the ratio of a red seabream and primary producers becomes 9. Since the nitrogen ratio is thought to be ncreased three when trophic level becomes higher by each one rank, the red sea-bream would be higher by three rank from primary producer. The carbon isotopic ratio of the red sea-bream is about -16. The ratios of phytoplankton and eelgrass, both primary producer are about -21 and -10, respectively. Therefore, the fish is estimated to get its carbon sources from both primary producers. The juvenile fish can utilize various food through three trophic levels containing fast primary producer and slower one in the flat. The interactions among these ecological diverse organisms are etimated to support the high and multiple production and the large purification in the tidal flat. So, we must protect these diverse organisms in a tidal flat in order to keep and develop sustainable fisheries.

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RECENT RESEARCH ON GENETIC IMPROVEMENT OF AQUACULTURED SPECIES WITH REFERENCE TO QUANTITATIVE OR POPULATION GENETICS IN JAPAN

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ABSTRACT

Government-supported projects on genetic improvement of aquacultured species in Japan are listed focusing to quantitative or population genetics. The history and results of studies on genetic improvement for the Japanese pearl oyster are summarized as examples of genetic work on intensive aquacultured species in Japan which has been conducted in the laboratory of the Japan Fisheries Agency.

INTRODUCTION

Japan has a long history of aquaculture in goldfish, carp, and oysters. For example, the xanthic (red) common carp seem to have first appeared in the early cultures of Europe, China, and Japan but reached its fame through the recent artificial selection of multicolored aberrants in the Niigata Prefecture of Japan.[1] Recently, however, the scientific technique has been developed to manipulate aquacultured species genetically in order to improve the quality of products. Development of the theories of genetics of quantitative traits and populations, cytogenetics, and modern molecular genetics has given a strong impact to research on the scientific breeding of aquacultured stocks. This paper describes briefly the outline of the current government-supported studies on genetic improvement of aquacultured species applying the theories of quantitative or population genetics in Japan.

BREEDING AND GENETIC DIVERSITY

Genetic diversity is important not only for the sustainable usage of aquatic natural biotic resources but also for the program of genetic improvement of aquacultured species. That is, the genetic resources should have a diversity as much as possible for the breeding programs, recombination and selection of genetic material in aquaculture so that future generations can respond to changes in the physical, biological, and economic environment. A breeding program substantially aims to change the average performance of a population in a desired direction to the benefit of industry and the consumer market. It is obvious that the goal of strategy of animal or plant breeding in aquaculture is much different from those of sea ranching and stock propagation or enhancement which may require more diversity for sustainable reproduction in the natural habitat.

TREND IN JAPAN

When we look into the international trend of research on aquaculture, many programs on aquaculture genetics have shown that selective breeding can improve fish or shellfish stock performance, but it has been difficult to implement breeding programs in many aquaculture industries in the world except for a few cases such as Atlantic salmon in Norway. Many aquaculturists have not been interested in the genetic improvement of quality, quantity of products, and productivity. In Japan, for the last decade, both national and many local governments seem to have been interested in financial support mainly for the research projects of cyto- or molecular genetics to improve aquacultured species. Many species for aquaculture have been studied in these fields in Japan, and triploid, gynogenesis, androgenesis and clones are believed to support the development of the genetic improvement techniques.[2,3] Recently the Japan Fisheries Agency has started large-scale subsidy grant programs on quantitative genetics (mainly selective and crossing breeding) for local government and college researchers in 1992 (Table 1). Six institutes of the Fisheries Agency, 25 prefectural research organizations, 11 colleges, and a software company have

Table 1. The research projects of breeding of aquatic organisms supported by the Fisheries Agency of Japan (1992–1996)

Name of project	Species	Number of organization involved
Marine finfish	Red sea bream (*Pagrus major*) Japanese flounder (*Paralichthys olivaceus*)	4
Salmonid fish	Rainbow trout (*Oncorhynchus mykiss*) Coho salmon (*O. kisutch*) Amago salmon (*O. masou ishikawae*) Japanese char (*Salivelinus pluvius*)	13
Freshwater fish	Ayu (*Plecoglossus altivelis*)	5
Pearl oyster	Japanese pearl oyster (*Pinctada fucata martensii*)	5
Abalone	Abalone (*Haliotis* (*Nordotis*) *discus hannai*)	4
Oyster	Pacific oyster (*Crassostrea gigas*)	3
Marine algae	Laver (*Porphyra tenera*, *P. yezoensis*)	4
Development of basic technology	Cherry salmon (*Oncorhynchus masou*) Many species of freshwater fish	6

been involved with this grand project. This plan included many traits of many species and many different types of researches to be conducted independently at many localities in Japan. The species are shellfishes like the Pacific oyster, the Japanese pearl oyster, abalone, and finfish like the red sea bream, the Japanese flounder, rainbow trout, the Amago salmon, the Japanese char, and the laver. Smaller-scale experimental breeding practices had been conducted at the private college or prefectural hatcheries. This subsidy grant project started focusing on the evaluation of the stocks bred in these hatcheries at the initial stage of the program.

Another government-supported project was initiated in 1995 in order to obtain basic information on the mechanism of sex determination of the fish larvae in the environment of the hatchery and the information on the effective number of parents for the fish and shellfish seed production to be released for sea ranching. The release of tremendous numbers of hatchery-produced fish or shellfish to the natural habitat has raised concern with regard to loss or biased changes of genetic diversity in wild populations of many species in Japan. This program includes the development of techniques for the evaluation of sex ratios at the earliest stage of fish larvae and genetic diversity of hatchery produced seeds for release in the natural habitat. The program also includes theoretical studies on the genetic impacts of released seeds on the populations in the natural ecosystem. The organizations supported by this grant are four laboratories in the Fisheries Agency, the Nippon Saibaikyokai (Japan Sea-Farming Association), three prefectural organizations and seven colleges.

CASE STUDY - PEARL OYSTER

As examples of the genetic works of aquacultured species in Japan, the history and results of studies on genetic improvement of economic traits of the cultured Japanese pearl oyster *Pinctada fucata martensii* are summarized. The breeding studies of the Japanese pearl oysters initiated around 1970 at the National Pearl Research Laboratory (which was reorganized into a part of the National Research Institute of Aquaculture (NRIA) in 1979) when some hatcheries started to provide seeds to the pearl industry. The traits studied were size and shape of shell (shell width and convexity), and color of shell (pearl and prismatic layers) (Table 2). The results showed these traits were heritable, which can be applied to the commercial hatcheries. The local governmental research organizations and private hatcheries have followed these results for large-scale breeding programs in each locality.

The yellow pigments in the pearl layer (nacre) of shell has been shown heritable and this was useful for the pearl industry. Most pearls contain yellow pigments in the nacre and they are less valuable than pearls with other colors in the current market. Experimental transplantation of the mantle tissue has suggested that yellow pearls were produced from grafting of the mantle tissue dissected from the shells with yellow nacre. Sib analysis and selection experiments showed that this trait was highly inherited.[5,6]

The external color of bivalve shells is mainly associated with pigments contained in the prismatic layer of the shells. White specimens which were rare in the wild population where the brown is common have recently become frequent in the artificial seeds. Mating experiments suggested that the white coloration is inherited under recessive gene (s). The trait is also useful for the production of pearls lacking yellow pigments because the amount of yellow pigment in the nacre of white prismatic shells was smaller than in the nacre of other color types.

Table 2. The heritability of quantitative traits in the Japanese pearl oyster *Pinctada fucata martensii*

Traits \ Methods	Selection Response	Sib Analysis
Color of nacre (yellow)	High	*
Size of shell (shell width)	Medium	Medium
Shape of shell (convexity)	Medium	Medium
Weight of shell (dried)	Medium	Medium
Larval shell growth	*	Low

* not estimated, Source : Wada[4, 6, 7, 8]

Other quantitative traits studied are shell width, shell convexity (shell width/ (shell length+shell height+shell width), and shell weight which have been shown moderately heritable (estimated heritabilities are 0.2-0.4) by the sib analysis and selection response. The average heritabilities of larval shell growth were estimated low (0.078-0.335).[7,8]

Spawning season or maturation cycle has been also suggested heritable from the histological observation of gonadal seasonal change among the different local races of this species which are distributed from northern temperate to southern tropical regions.[9]

Regarding the conservation of genetic diversity, the perspectives of strategy for genetic conservation of the species have been proposed to promote the breeding and to preserve various genotypes in wild populations (Figure 1).[4] The seeds of the pearl oysters have been collected from the wild set since World War II using a ceder leave collector. Before that time women divers used to collect the mother of pearl for the operation to produce pearls. Currently, both natural and artificial seeds are still used and the numbers of hatchery seeds are increasing. The hatchery of this species began around 1960 to supplement decreased or extinct seed production in some regions and to attempt selective breeding. The artificial seeds are produced mostly in restricted areas where the natural seed is

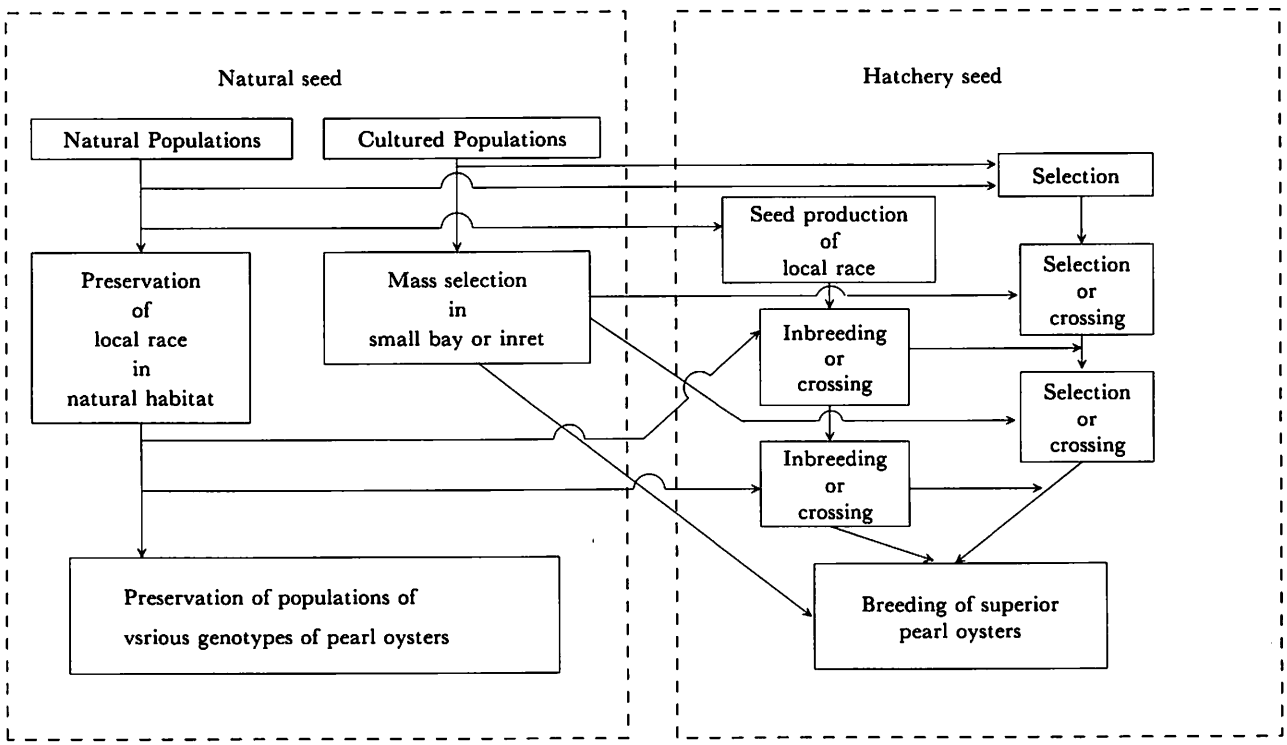

Figure 1. Proposed strategy for genetic conservation and breeding for the intensive culture of the Japanese pearl oyster in Japan (Wada[4])

difficult to collect, which has a benefit to avoid the loss of genetic diversity in the natural population. The preservation of various genotypes should be considered when the seeds from the hatchery are introduced into the area where natural populations are still abundant. Genetic diversity is very important for the breeding program which has to respond to the various demands of farmers or consumers with regard to biological, physiological, ecological or economical changes in the future.

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THE USE OF CAPTIVE BROODSTOCKS FOR GENE CONSERVATION OF SALMON IN THE WESTERN UNITED STATES

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ABSTRACT

Captive broodstock programs are an important tool for the genetic preservation and recovery of threatened or endangered species. The high fecundity of Pacific salmon, coupled with their potentially high survival in protective culture, affords an opportunity for captive broodstocks to produce large numbers of juveniles in a single generation for supplementation of natural salmon populations. Considerable experience in ocean ranching of Pacific salmon and in hatchery production of juvenile fish has provided the technological base for culture of salmonids during early development. However, factors affecting growth, development, and maturation later in the salmon life cycle are poorly understood. In this paper, we review captive broodstock projects that have been initiated for some of the most depressed stocks of chinook salmon (*Oncorhynchus tshawytscha*) and sockeye salmon (*O. nerka*) in the Pacific Northwest. In general, egg-to-adult survival rates for Pacific salmon captive broodstocks have ranged from 30-40%. Viability of eggs from captively-reared spawners has been commonly only 30-60% compared to viabilities of over 80% for the eggs from wild cohorts. In addition, initial field trials indicate that wild coho salmon had greater estimated breeding success than comingling captively-reared pairings. Furthermore, the size of captively-reared adults has been generally smaller than that of wild fish. Nonetheless, Pacific salmon captive broodstocks have been successful in producing 100-1000 times more progeny than would have been available if the endangered populations had not been taken into captivity. In concert with efforts to correct causes of the decline of stocks at risk of extinction, captive broodstock technology holds promise as a means of accelerating stock recovery by rapidly increasing the abundance of fish available for restocking suitable habitat.

INTRODUCTION

Several stocks of anadromous salmonids in the Pacific Northwest are listed as threatened or endangered under the U.S. Endangered Species Act (ESA).[1-3] In addition, over 200 stocks have been identified as "of special concern."[4] The National Marine Fisheries Service (NMFS) is developing recovery plans for ESA-listed stocks of Pacific salmon.[5] The ESA recognizes that conservation of listed species may be facilitated by artificial means, such as captive broodstocks, while factors impeding population recovery are identified and corrected.[6]

Two captive broodstock approaches are being applied to salmon recovery in the Pacific Northwest. One strategy involves capturing wild prespawning adults, fertilized eggs, or juveniles from their native habitats, and rearing the populations to maturity in hatcheries. The first or second generation offspring are then stocked into ancestral lakes or streams at one or more juvenile life stages (e.g., fry, parr, smolt). Another strategy involves rearing the broodstock in captivity to adulthood, then releasing the adults back into their natal habitats to spawn naturally.

Pragmatically, captive broodstocks may offer the best chance for continued existence of endangered populations through enhanced survival during protective culture.[7] Considerable experience in ocean ranching of Pacific salmon and in hatchery production of juvenile fish has provided the technological base for culture of salmonids during early development. However, relatively few previous attempts have been made to grow anadromous Pacific salmon to maturity in captivity,[8] and little is known regarding specific techniques to maximize survival and reproduction. Realistic criteria defining success of captive broodstocks are as yet undefined.

In this paper, we review some of the captive broodstock projects that have been conducted for depressed stocks of

chinook (*Oncorhynchus tshawytscha*), sockeye (*O. nerka*), and coho salmon (*O. kisutch*) in the Pacific Northwest. Information from these projects is used to develop a basis for establishing expected survival and reproductive performance for future salmonid captive broodstock efforts.

METHODS

Data from published and unpublished reports, as well as information obtained in interviews with biologists involved in captive rearing of Pacific salmon were compiled on survival, reproductive success, and offspring fitness of captive broodstocks. This effort concentrated on the five formal captive broodstocks that are currently underway to aid recovery of anadromous Pacific salmon stocks in the Pacific Northwest: (1) Snake River sockeye salmon from Redfish Lake (Idaho), listed as endangered under ESA; [9-12] (2) Snake River spring/summer chinook salmon from the Salmon (Idaho) and Grande Ronde (Oregon) River Basins, listed as threatened under ESA (T. Flagg, NMFS, unpubl. data); (3) White River (Washington) spring chinook salmon, identified by the state of Washington as a stock of concern;[13] (4) Dungeness River (Washington) spring chinook salmon, identified by the state of Washington as a stock of concern;[14] and (5) Hood Canal (Washington) coho salmon, identified by the state of Washington as a stock of concern.[15]

Information was also obtained from a number of other public and private programs that have used captive broodstocks to maintain egg supplies for selected stocks of fish. For instance, NMFS has reared over a dozen other stocks and year-classes of Pacific salmon to maturity at the Manchester Marine Experimental Station near Manchester, Washington USA.[8] Fishery agencies in Washington, Alaska, and Canada have also used captive broodstock technology for stock enhancement. Preliminary results of NMFS behavioral interactions research for captively-reared and wild fish were also reviewed.

RESULTS AND DISCUSSION

The dramatic difference between the natural environments experienced by wild Pacific salmon and the artificial environments experienced by captively-reared fish appears to create a number of differences in their relative reproductive potential. In general, captively-reared Pacific salmon appear less reproductively fit than their wild cohorts.

The size and age of maturity of captively-reared adults were generally less than wild cohorts. For instance, Joyce et al.[16] indicated that captively-reared Unuk River, Alaska, chinook salmon females matured at 4 yr of age and 6.8 kg (Table 1), while wild cohorts matured at 5 yr and 12.8 kg. Similarly, Flagg et al.[12] reported that captively-reared Redfish Lake sockeye salmon matured at 3 yr of age and 1.2 kg compared to 4 yr and 2.0-3.0 kg for wild fish (K. Johnson, Idaho State Department of Fish and Game (IDFG), pers. commun.). Part of this size discrepancy can be attributed to early maturity of captively-reared fish. However, even in cases where age at maturity of captively-reared fish mimicked wild fish (e.g., Lake Wenatchee sockeye salmon; Table 1), their size was generally 20-50% less than wild stock.[17-18] Average viability of eggs from captively-reared spawners (30-70%) was also commonly lower than the 75-95% viability of similar strains of hatchery-spawned wild fish.[18-19]

The reasons for the generally poorer reproductive performance of artificially propagated captively-reared fish compared to ocean ranched and wild cohorts are not well understood. Most captive broodstock programs we reviewed used spawners collected from the wild population. Therefore, it seems intuitive that much of the poor performance, at least in first-generation offspring, can be attributed to the effects of artificial culture environments. Unfortunately, husbandry records in most of the references cited in Table 1 did not allow a systematic investigation of the reasons for reduced reproductive success of the captively-reared fish. We speculate that development of nutritionally complete species-specific brood diets would improve reproductive performance of captive broodstocks by improving the gametes quality. However, the effects of genetic change in the captively-reared populations as a basis for reduced spawner size, egg viability, and reproductive behavior of fish remain a possibility.[20]

Preliminary results from recent behavioral studies indicate that captively-reared Pacific salmon released to spawn in streams may also have lower breeding success than comingling wild coho salmon (B. Berejikian, NMFS, unpubl. data). In this study, equal-sized captively-reared 1992-brood Hood Canal coho salmon (Table 1) and wild coho salmon were released into duplicate spawning channels. In these spawning aggregates, wild males spawned with females from both populations more frequently than did captively-reared males (Table 2), indicating that wild males dominated competition for sexually-active females. Females from both wild and captively-reared populations preferred wild rather than captively-reared males. Finally, wild females established nesting territories earlier, and constructed more nests per individual, than captively-reared females, suggesting a competitive advantage for wild females (Table 2). Although they had lower breeding success than wild salmon, captively-reared coho salmon did demonstrate the full range of coho salmon reproductive behaviors and the ability to naturally reproduce.

The morphology and body coloration of wild and captively-reared adults used in this experiment differed markedly, and may have contributed to the differences in reproductive success. Other studies have demonstrated

Table 1. Rearing history, survival during culture, and reproductive success of selected captive broodstocks of sockeye (*Oncorhynchus nerka*), chinook (*O. tshawytscha*), and coho salmon (*O. kisutch*).

Brood year/stock	Reared from[a]	Culture site[b]	Survival to adult (%)	Major disease problem[c]	Average female spawner: Age (years)	Size (kg)	Fecundity	Eyed-egg viability (%)	Reference[d]
Sockeye salmon:									
1987 Auke Lake, AK	age 0	SWP[e]	58	nd	4	1.2	nd[f]	10	W. Heard, NMFS, unpubl. data
1988 Lake Wenatchee, WA	age 1	SWP	0	BKD	-	---	----	–	C. McAuley, NMFS, unpubl. data
1989 Redfish Lake, ID	age 1	FWT	20	BKD, Aerom.	5	3.2	2,150	29	K. Johnson, IDFG, unpubl. data
1990 Redfish Lake, ID	age 1	FWT	20	Aerom.	4	2.8	1,750	20	K. Johnson, IDFG, unpubl. data
1990 Lake Wenatchee, WA	age 1	FWT	32	BKD	4	2.2	2,477	50	Flagg et al. 1996[12]
1990 Lake Wenatchee, WA	age 1	SWT[g]	35	BKD	4	1.6	1,899	42	Flagg et al. 1996[12]
1990 Lake Wenatchee, WA	age 1	SWP[g]	26	BKD	4	1.0	1,703	46	Flagg et al. 1996[12]
1991 Lake Wenatchee, WA	age 1	FWT	88	BKD	4	1.5	2,206	67	T. Flagg, NMFS, unpubl. data
1991 Lake Wenatchee, WA	age 1	SWT[g]	61	BKD	4	1.1	1,849	43	T. Flagg, NMFS, unpubl. data
1991 Lake Wenatchee, WA	age 1	SWP[g]	26	BKD	4	0.8	1,561	40	T. Flagg, NMFS, unpubl. data
1991 Redfish Lake, ID	egg	FWT	84	none	3	2.2	1,995	22	K. Johnson, IDFG, unpubl. data
1991 Redfish Lake, ID	egg	FWT	21	BKD	3	1.2	1,644	60	T. Flagg, NMFS, unpubl. data
1992 Redfish Lake, ID	egg	FWT	77	none	2	1.6	1,600	48	K. Johnson, IDFG, unpubl. data
1993 Redfish Lake, ID	egg	FWT	86	none	3	2.4	1,980	63	K. Johnson, IDFG, unpubl. data
1993 Redfish Lake, ID	egg	FWT	84	none	3	1.2	1,400	69	C. McAuley, NMFS, unpubl. data[h]
1993 Redfish Lake, ID	egg	SWT[g]	76	none	3	1.0	1,249	79	C. McAuley, NMFS, unpubl. data
Chinook salmon:									
1977 White River, WA	age 1	SWP[g]	3	BKD, MIA	5	nd	nd	nd	L. Harrell, NMFS, unpubl. data
1980 White River, WA	age 1	SWP[g]	7	BKD, MIA	4	nd	nd	32	L. Harrell, NMFS, unpubl. data
1980-82 Snake River, WA	age 0	SWP[g]	2-4	BKD, Rosette	4	1.5-2.0	nd	0	Harrell et al. 1987[30]
1984 Unuk River, AK	age 1	SWP[g]	<10	nd	4	6.8	nd	72	Joyce et al. 1993[16]
1984 Squamish River, BC	age 1	SWP[g]	8	BKD	3	5.0	4,069	65	Fedorenko and Cross 1991[31]
1985 Squamish River, BC	age 1	SWP[g]	4	BKD	3	4.4	4,341	87	Fedorenko and Cross 1991[31]
1987-89 White River, WA	age 1	SWP[g]	23-29	Vibrio, BKD	4	3.0-7.0	<2,700	<70	Appleby and Keown 1995[13]
1993 Dungeness River, WA	age 0	FWT	78	none	4	4.3	3,800	75	D. Witczak, WDFW, unpubl. data
Coho salmon:									
1978 DOMSEA domestic, WA	age 1	SWP[e]	23	furunculosis		nd	nd	45	McAuley 1981a, b[32,33]
1980-85 DOMSEA domestic, WA	egg	SWP[g]	3-5	BKD	2	1.5	2,700	82	C. McAuley, NMFS, unpubl. data
1980-85 DOMSEA domestic, WA	egg	FWT	50-60	nd	2	3.0	4,000	>85	C. McAuley, NMFS, unpubl. data
1992 Hood Canal, WA	age 0	FWT	>80	none	3	1.4	1,725	45	S. Schroder, WDFW, unpubl. data

[a] N=300-3,000+

[b] SWP=seawater net-pen, FWT=circular tank supplied with fresh (well) water, SWT=circular tank supplied with filtered and UV-sterilized seawater. All fish reared in SWT and SWP groups were reared from egg-smolt in freshwater.

[c] BKD=bacterial kidney disease caused *Renibacterium salmoninarum*; Aerom.=diseases caused by motile aeromoands, *Aeromonas sp.*; MIA=marine infectious anemia caused by *Enterocytozoon salmonis*; Rosette disease caused by a systemic obligate intercellular eukaryotic pathogen;[30] Vibrio caused by *Vibrio anguillarum*; furunculosis caused by *Aeromonas salmonicida*. Note: programs also documented losses due to mechanical failures, unaccounted for inventory discrepancies, and, in SWP groups, predation by marine mammals.

[d] NMFS=National Marine Fisheries Service, USA; IDFG=State of Idaho Department of Fish and Wildlife, USA; WDFW=State of Washington Department of Fisheries and Wildlife, USA.

[e] Fish spawned directly from seawater.

[f] nd=no data available in Reference.

[g] Fish returned to freshwater for spawning.

[h] Data through December 1996 with approximately 80 per cent of 500 females eggs eyed.

Table 2. Summary of reproductive behavior and success of comingling captively-reared and wild coho salmon in an experimental stream channel (Berejikian, unpubl. data).

Sex	Attribute	Wild	Captively-reared
Males			
	Access to females	Greater	Less
	Courtship behavior	High levels	Low levels
	Reproductive success	High	Low
Females			
	Number nests/female	Many	Few
	Onset of spawning	Early	Late
	Egg retention	Low	High
	Reproductive success	High	Low

that hatchery rearing of Pacific salmon can result in phenotypic divergence from the wild state for a variety of morphological characters including secondary sex characteristics,[21] which play an important role in the reproductive behavior and success of salmon on the spawning grounds.[22-26] Coloration patterns are used as status signals in agonistic encounters of juvenile salmonids,[27-28] adult chum salmon,[22] and are believed to be a factor for adult sockeye salmon.[29] These apparent deficiencies in captively-reared adult salmon might be minimized by improved feeds (e.g., increasing carotenoid levels) and providing exercise for improved physiological development.

Survival during captive broodstock culture ranged from 0-88% for sockeye salmon, 2-78% for chinook salmon, and up to 80%+ for coho salmon (Table 1). A multitude of factors including rearing environment and health status can influence survival. Generally, average survival during culture appeared higher for sockeye and coho salmon than for chinook salmon. However, husbandry records did not allow exact determination of the reasons for the differences among species. The major disease problem noted in most captive broodstock programs was bacterial kidney disease, caused by *Renibacterium salmoninarum* (Table 1). However, this problem was reduced when fish were cultured in water with low concentrations of pathogens (e.g., fresh well water or filtered and sterilized seawater) rather than in seawater net-pens. Captive broodstock culture in these reduced pathogen environments should be considered for future programs.

Although in many cases, average survival and eyed-egg viability of captive broodstocks have not met expectations, they still are fulfilling most supplementation goals. For instance, 1991-brood Redfish Lake sockeye salmon captive broodstocks (Table 1) were established from capture of the only female and three male fish that returned to the lake that year. The low survival and eyed-egg viability of combined NMFS/IDFG captive broodstocks resulted in production of less than 60% of projections for that group. Nevertheless, the 1991-brood captive broodstock produced over 90,000 juveniles released in Redfish Lake in 1995 (T. Flagg, NMFS, and K. Johnson, IDFG, unpubl. data). The captive broodstock of 1993-brood Redfish Lake sockeye salmon (Table 1) was founded from two female and six males and is projected to produce up to 500,000 juveniles for ESA recovery efforts. Similarly, White River captive broodstocks are now producing almost a million eggs a year for enhancement (K. Keown, Washington State Department of Fisheries and Wildlife, pers. commun.). These population amplifications represent 100-1000 times the current estimated natural egg-to-adult survivals in these endangered stocks.

Presently, little is known regarding survival of juveniles released from captive broodstocks. Monitoring and evaluation of past captive broodstock programs has not been extensive. We know of only one case where survival to adult has been documented for progeny of captive reared fish released to the wild. Progeny of 1984-brood Squamish River, B.C., Canada, captive broodstock (Table 1) were reared in a hatchery, released to the ocean, and survival compared to progeny of ocean-ranched fish. In this study, average smolt-to-adult survival for groups of progeny from captive reared parents was 2.2% compared to 3.4% for progeny of normal ocean-ranched parents (E. Perry, Canada Department of Fisheries and Oceans, pers. commun.). During the next few years, several ongoing captive broodstock programs (e.g., Redfish Lake sockeye salmon, White River chinook salmon, and Dungeness River chinook salmon) will provide smolt-to-adult survival information that should help define risks and benefits.

CONCLUSIONS AND RECOMMENDATIONS

We conclude that captive broodstock technology for salmonids, although in its initial development stages, is sufficiently advanced to allow carefully planned programs. In general, fishery managers can anticipate survivals of 50-80% in captive broodstocks if the fish are cultured in water sources low in pathogens. Viability of eggs from captively-reared spawners should range from 30-70%. The size and age at maturity for captively-reared fish will typically be less than wild fish, and the success of supplementation using offspring from captive broodstocks is for the most part untried and uncertain.

Since a multitude of factors affect both the decline and potential for recovery of a stock, exacting rules cannot be developed to define conditions for implementation of captive broodstocks. However, in general, use of captive broodstocks should be restricted to situations where the natural population is dangerously close to extinction.

Proper precautions should be taken to minimize genetic impacts during the collection, mating, and rearing of captive broodstocks, as any alteration to the original genetic composition of the population in captivity may reduce the efficacy of supplementation in rebuilding the natural population. Furthermore, liberation of fish from captive broodstocks should be consistent with the behavior of existing wild fish, or on knowledge of the life-history characteristics of the wild fish if none remain.

Captive broodstocks should be viewed as a short-term measure to aid in recovery--never as a substitute for reestablishing naturally spawning fish in the ecosystem. Because the benefits and risks have not been established through long-term monitoring and evaluation, captive broodstock development should be considered an experimental approach and used with caution. Captive broodstocks can provide an egg-base to help "jump-start" a population, but these efforts must go hand-in-hand with scientifically-sound resource management (e.g., habitat restoration, harvest reform) to fully aid in recovery. Primary consideration should be to restoring fish in the habitat. However, in some cases captive broodstocks may provide the only mechanism to prevent extinction of a stock and may be undertaken regardless of prospects for immediate habitat improvement.

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GENETIC EVALUATION OF QUANTITATIVE AND QUALITATIVE TRAITS OF HATCHERY STOCKS FOR AQUACULTURE IN RED SEA BREAM

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ABSTRACT

Qualitative and quantitative traits of the five hatchery stocks for put-and-take fisheries and net-cage culture in red sea bream, *Pagrus major* were evaluated using offspring which were propagated under the same rearing conditions. Body weight and length of selective breeding stocks were significantly larger than those of a non-selective breeding stock. Significant difference among the five stocks was also found in the morphological traits such as body depth and orbit diameter.

Distinct genetic divergence was observed among these hatchery stocks especially in the stocks exposed by the persistent and successive selection on growth traits. The number of alleles per one isozyme locus was remarkably decreased in hatchery stocks comparing with the wild population. Drastic decrease of allele number was also recognized in the hyper-variable DNA markers such as mini- and micro-satellite loci. However, the average heterozygosity in hatchery stocks did not decrease comparing with the wild stock in the isozymic and DNA markers.

The hatchery stocks examined in this study were recognized as a selective breeding line being excellent in performance traits for net-cage culture of red sea bream. The decrease of alleles per locus in these hatchery stocks may be caused simply by bottle neck effects, but not always be affected by inbreeding since the average heterogygosity of these stocks were maintained in high level.

INTRODUCTION

In recent 20 years, aquaculture production of red sea bream (*Pagrus major*=*Pagrus auratus*) has rapidly increased to 73 thousand tons in 1993.[1] As the demand of red sea bream fish seed increased for net-cage culture as well as put-and-take fisheries, the numbers of seed fish produced in private hatcheries also increased to about 100 million fish in 1993. Private hatcheries tend to develop and produce genetically improved seeds by selective breeding. However, there is no evidence of genetically improvement in these stocks produced in private hatcheries. We compared the average body sizes of several stocks propagated under the same rearing conditions,[2] and also examined genetic traits such as isozymes and DNA polymorphic markers.[3] In this paper, the present state of genetic change and improvement are evaluated both in quantitative and qualitative traits of red sea bream stocks which are preferred by farmers as a seed fish for net-cage culture.

MATERIALS AND METHODS

Genetic background of stocks used

Five hatchery stocks were used in this study (Table 1). These hatchery stocks consist of one non-selective breeding line for release to enhance natural population (stock 1) and four successive selective breeding lines for net-cage culture (stocks 2 to 5). Genetic backgrounds and origins are shown in Table 1. One sample from a natural population in Tosa Bay in Kochi Prefecture was also used to compare genetic traits such as DNA fingerprinting and microsatellite DNA polymorphism.

Rearing conditions

Ten grams (about 18,000 eggs) of fertilized eggs were collected from each hatchery at the same day and year. The eggs were transported within 24 hours after fertilization to the Experimental Station of Kochi Prefecture by car or aircraft using plastic bags. The fertilized eggs were placed into the incubation tanks for hatching. About 13,000 to 16,000 hatched larvae were placed into separate

800 liter FRP (fiber reinforced plastic) tanks.

During 60-day larval and juvenile stages, rotifer and artemia were used as live food. Sixty days after hatching, environmental conditions such as density, feeding, water supply, and water temperature were adjusted in five FRP tanks for rearing experiments. Samples were collected for measurements of body length and weight in the 3, 6, 10, 15, 30, 50, 70, 100, 150, and 200 day old fish. Samples collected were also used for isozyme and DNA analysis.

Detection of isozyme and DNA polymorphism

Isozyme patterns were detected by horizontal starch-gel electrophoresis using skeletal muscle and hepatic tissue.[4] Genomic DNA was extracted from blood cell. The genome DNA digested by Hinf-I was separated by acrylaminde electrophoresis and visualized by hybridizing with radiolabelled 33.15 probe.[3] The microsatellite DNA (GT/CA) fragments were amplified by primers developed by Takagi et al.[5] The fragments were separated by acrylaminde electrophoresis, and visualized by RI.[5]

RESULTS

Comparisons of mean values of body size and weight among hatchery stocks

A distinct difference among stocks appeared in the mean values of body length and body weight (Figure 1) in the first 50 days of rearing. The selective breeding stocks 2-5 showed better performances than the non-selective stock 1. These differences were observed consistently from 3-day-old to 200-day-old individuals (Figure 2). The maximum difference between stocks at 200 days old reached 46 g (about 56.7% larger) in mean body weight and 22mm (about 26.8% bigger) in mean fork length (Table 2).

Change in genetic variability and divergence in allele frequencies

Genetic variability

Isozymes. Genetic variability of the five hatchery stocks was examined based on the 17 isozyme loci (Table 3). No distinct difference was found in the average number of alleles per locus and the average heterozygosity between

Table 1. Genetic background * of the hatchery stocks of red sea bream examined in this study

Designation	The origin of each stock
Stock 1	The 2nd generation, by non-selective breeding, originated from a natural population from Kochi and Tokushima Pref.
Stock 2	The 3rd generation, by selective breeding in size, originated from the 4th generation of strain 3
Stock 3	The 7th generation, by selective breeding in size, originated from natural population of Wakayama Pref.
Stock 4	The 2nd generation, by selective breeding in size, originated from a natural population of Fukuoka Pref.
Stock 5	The 4th generation, by selective breeding in size, originated from the cross breeding between Korean and Japanese natural populations

* Precise information about selection method, and selection intensity were not available.

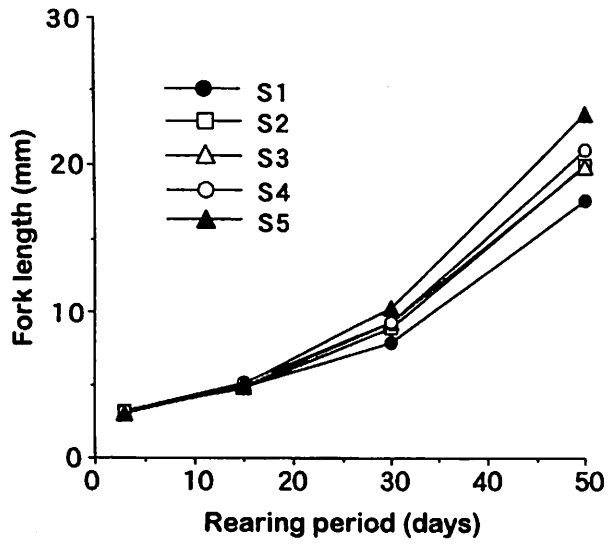

Figure 1. Comparison of growth curves in fork length for 5 hatchery stocks of red sea bream from hatching to 50 days old.

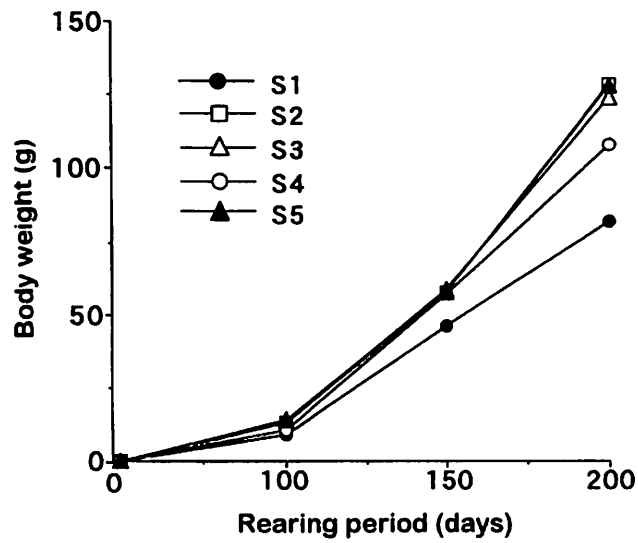

Figure 2. Comparison of growth curves in body weight for 5 hatchery stocks of red sea bream from hatching to 200 days old.

Table 2. Mean and standard deviation (SD) of body length and weight for 5 stocks of red sea bream with statistical analysis* in each sampling day

	S1 (a)	S2 (b)	S3 (c)	S4 (d)	S5 (e)
Total length (mm)					
3days	3.09 ± 0.24^{d}	3.17 ± 0.14^{e}	3.13 ± 0.17	3.23 ± 0.15^{ae}	3.03 ± 0.26^{bd}
6days	3.32 ± 0.11^{d}	3.37 ± 0.13^{cd}	3.28 ± 0.11^{bd}	3.48 ± 0.14^{abce}	3.33 ± 0.15^{d}
10days	3.53 ± 0.25^{bcd}	3.75 ± 0.23^{ad}	3.71 ± 0.20^{ad}	4.02 ± 0.24^{abce}	3.67 ± 0.28^{d}
15days	4.92 ± 0.37	4.80 ± 0.45^{de}	4.93 ± 0.44	5.16 ± 0.42^{b}	5.08 ± 0.33^{b}
30days	7.92 ± 1.04^{bcde}	8.85 ± 1.07^{ae}	9.31 ± 1.38^{ae}	9.29 ± 1.26^{ae}	10.3 ± 1.49^{abcd}
Fork length (mm)					
50days	17.6 ± 2.38^{bcde}	20.0 ± 2.85^{ae}	19.9 ± 2.28^{ae}	21.0 ± 2.79^{ae}	23.4 ± 2.44^{abcd}
70days	37.9 ± 7.55^{ce}	42.3 ± 6.44	41.0 ± 7.42^{a}	40.4 ± 7.59	43.2 ± 5.76^{a}
100days	70.4 ± 7.28^{bce}	80.6 ± 6.89^{ad}	81.3 ± 6.50^{ad}	71.8 ± 8.76^{bce}	80.5 ± 7.66^{ad}
150days	125.1 ± 9.38^{bcde}	134.2 ± 8.54^{a}	134.6 ± 7.41^{a}	131.3 ± 10.89^{a}	134.1 ± 7.92^{a}
200days	149.8 ± 10.25^{bcde}	170.7 ± 9.58^{ad}	169.5 ± 7.67^{ad}	160.2 ± 8.94^{abce}	171.6 ± 8.14^{ad}
Body weight (g)					
100days	8.97 ± 3.00^{bce}	12.82 ± 3.45^{ad}	13.34 ± 3.03^{ad}	10.28 ± 3.88^{bce}	13.75 ± 3.92^{ad}
150days	45.9 ± 11.57^{bcde}	57.3 ± 11.49^{a}	58.5 ± 10.12^{a}	57.0 ± 13.31^{a}	57.3 ± 10.06^{a}
200days	81.88 ± 18.75^{bcde}	128.3 ± 21.51^{ad}	123.7 ± 16.11^{ad}	107.6 ± 16.26^{abce}	127.4 ± 17.17^{ad}

* Stocks 1-5 were designated by a-e for analysis of variance. Superscripts shows significant difference between stocks ($P<0.01$).

the natural population and the hatchery stocks (Table 4). However, the minor alleles observed in the natural population were lost in the hatchery population as shown in proportion to polymorphic loci (Table 4).

DNA fingerprints. Figure 3 is an example of the electrophoretic pattern of hypervariable minisatellite DNA detected by probe 33.15 and Hinf I endonuclease. Genetic variability of the five hatchery stocks was estimated based on band sharing indices (BSI) from the so-called DNA fingerprinting (Table 5). Mean of BSI within a stock can be seen as an indicator of genetic variability of the stock. Mean BSIs were relatively high in hatchery stocks 2 and 3, low in the natural population, and intermediate in stocks 1, 4, 5 (Table 6). These values suggest that the genetic variability was partly lost in hatchery stocks.

Microsatellite DNA loci. Figure 4 is an example of the electrophoretic pattern of hypervariable microsatellite DNA which were amplified using a specific primer, Pma-4.[5] Drastic reduction in the number of alleles per locus was observed in hatchery stocks compared with the natural population (Figure 5). Average heterozygosity was also reduced in the hatchery population (Figure 6). The loss of genetic variation was well monitored by microsatellite markers as shown in Figures 5 and 6.

As the average heterozygosities of hatchery stocks were maintained in relatively higher condition in both isozyme and microsatellite loci in comparison with that of the natural population, it is suggested that these genetic changes may be caused mainly by random genetic drift, and not by inbreeding. Actually, larger numbers of parental fish have been used in mass production of seed fish especially in private hatcheries. Genetic divergence

Based on the 17 isozyme loci (Table 3), genetic distances were calculated using the Nei's formula.[6] Selective breeding stocks 2 and 3 being 7th generation were well diverged from other stocks and the natural population,[4] as shown in Figure 7 These genetic divergence might have appeared unconsciously by selection of size during artificial propagation.

DISCUSSION

Genetic improvement of growth performance in selective breeding stocks

The present state of hatchery stocks was evaluated based on the growth performance and genetic variability. Distinct genetic improvement of body length and size may be achieved consciously in selective breeding stocks 2-5 as shown in Table 2. The reduction of genetic variability in the isozymes and DNA polymorphism could be the consequence of the successive size selection for the 2nd to 7th generations.

Deficiency of growth traits in a non-selective breeding stock

The stock 1 being non-selective breeding line showed reduction of body length and weight. Since the stock 1 is maintained to produce seed fish for put-and-take fisheries, any selection for genetic improvement has not been done. The size of stock 1, however, is the smallest in body length

Table 3. Allele frequencies of polymorphic loci in 5 hatchery stocks and one natural population (NP) of red sea bream

Locus	Allele	S1	S2	S3	S4	S5	NP
*AAT-2**	*A*	0.000	0.000	0.000	0.067	0.000	0.015
	B	0.989	0.822	0.900	0.889	0.878	0.974
	C	0.011	0.178	0.100	0.044	0.122	0.011
*AAT-3**	*A*	0.000	0.000	0.000	0.000	0.000	0.003
	B	1.000	1.000	1.000	0.989	1.000	0.997
	C	0.000	0.000	0.000	0.011	0.000	0.000
*ADH**	*A*	0.156	0.244	0.167	0.256	0.222	0.248
	B	0.433	0.211	0.222	0.556	0.356	0.511
	C	0.411	0.544	0.611	0.189	0.422	0.241
*α GPD-1**	*A*	0.000	0.000	0.000	0.000	0.000	0.062
	B	1.000	1.000	1.000	1.000	1.000	0.938
	C	0.000	0.000	0.000	0.000	0.000	0.000
*EST**	*B*	0.000	0.000	0.000	0.000	0.022	0.000
	C	0.011	0.089	0.244	0.078	0.056	0.073
	D	0.111	0.000	0.000	0.078	0.100	0.168
	E	0.711	0.722	0.511	0.611	0.367	0.452
	F	0.133	0.167	0.111	0.089	0.289	0.263
	G	0.022	0.022	0.089	0.144	0.133	0.044
	H	0.011	0.000	0.044	0.000	0.033	0.000
*GPI-1**	*A*	0.000	0.000	0.000	0.000	0.000	0.033
	B	1.000	1.000	1.000	1.000	0.989	0.956
	C	0.000	0.000	0.000	0.000	0.011	0.011
*GPI-2**	*A*	1.000	1.000	1.000	1.000	1.000	0.997
	B	0.000	0.000	0.000	0.000	0.000	0.003
*IDH-1**	*A*	0.000	0.000	0.000	0.033	0.000	0.007
	B	1.000	1.000	1.000	0.967	1.000	0.993
	C	0.000	0.000	0.000	0.000	0.000	0.000
*IDH-2**	*A*	0.000	0.000	0.000	0.025	0.000	0.000
	B	1.000	1.000	1.000	0.975	1.000	0.997
	C	0.000	0.000	0.000	0.000	0.000	0.003
*LDH-2**	*A*	0.000	0.000	0.000	0.000	0.000	0.003
	B	1.000	1.000	1.000	1.000	1.000	0.997
	C	0.000	0.000	0.000	0.000	0.000	0.000
*MDH-1**	*A*	0.000	0.000	0.000	0.011	0.000	0.000
	B	1.000	1.000	1.000	0.978	1.000	0.978
	C	0.000	0.000	0.000	0.011	0.000	0.022
*MDH-2**	*A*	0.000	0.000	0.000	0.000	0.000	0.003
	B	1.000	1.000	1.000	1.000	1.000	0.997
	C	0.000	0.000	0.000	0.000	0.000	0.000
*ME-1**	*A*	0.356	0.244	0.159	0.367	0.256	0.358
	B	0.478	0.356	0.511	0.567	0.567	0.471
	C	0.078	0.056	0.023	0.011	0.111	0.095
	D	0.089	0.344	0.307	0.056	0.167	0.077
*PGM-1**	*A*	0.951	0.963	0.939	0.939	0.978	0.854
	B	0.049	0.037	0.061	0.061	0.022	0.142
	C	0.000	0.000	0.000	0.000	0.000	0.004
*PGM-2**	*A*	0.000	0.000	0.000	0.011	0.000	0.004
	B	1.000	1.000	1.000	0.989	1.000	0.996
	C	0.000	0.000	0.000	0.000	0.000	0.000
*6PGD**	*A*	0.167	0.133	0.122	0.167	0.189	0.146
	B	0.833	0.867	0.878	0.833	0.789	0.843
	C	0.000	0.000	0.000	0.000	0.011	0.011
*SDH**	*A*	0.033	0.011	0.000	0.011	0.022	0.062
	B	0.967	0.989	1.000	0.989	0.978	0.938
	C	0.000	0.000	0.000	0.000	0.000	0.000

NP: Natural population collected from Tosa Bay (Taniguchi and Sugama, 1990)

33.15, Hinf I

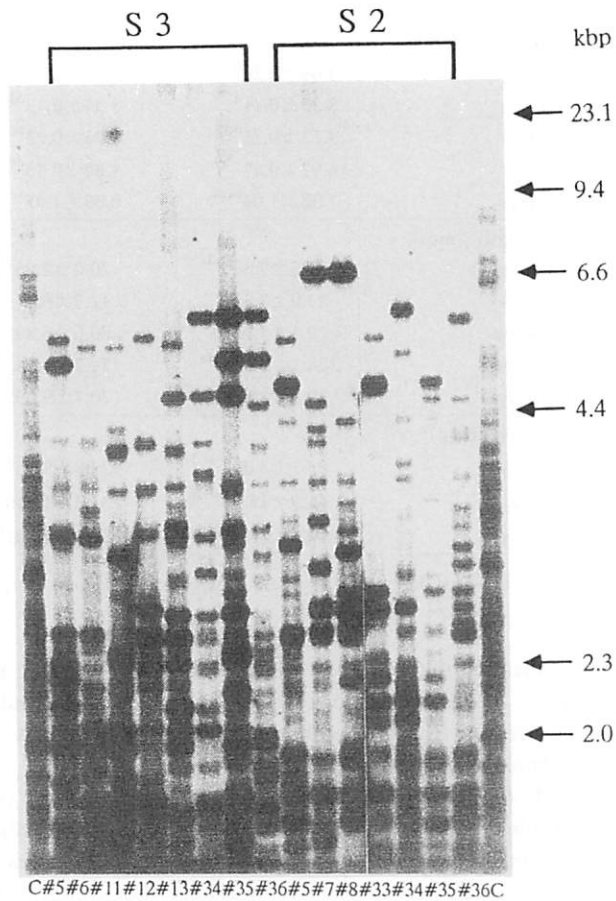

Figure 3. An example of DNA fingerprinting of hatchery stocks of red sea bream using gnome DNA digested by Hinf-I and hybridized with radiolabelled 33.15 probe. Counting from the left, lanes 2-9 are from hatchery stock 3, and lanes 10-16, from hatchery stock 2.

in comparison with hatchery stocks 2-5. If the growth performance of non-selective breeding stock 1 is inferior to that of the natural population, this is also a kind of so-called unconscious selection. If the non-selective line is inferior to that of the natural population as a seed fish in put-and-take fisheries, a decrease in the productivity in the mixed population of hatchery stocks and the natural one is possible.

Domestication in hatchery stocks

The non-selective stock examined tended to show sensitive behavioral responses and to evade to signs of human presence and other sounds around the rearing tanks. However, the selective breeding stocks tended to gather in the direction of the person feeding them. These phenomena may be a kind of domestication gained through unconscious selective behavior.

Table 4. Estimated parameters of genetic variability for hatchery stocks of sea bream strains based on 17 loci examined

Stocks	Average number of alleles per locus	Proportion of polymorphic loci		Average heterozygosity		
		P1	P2*2	H_o	H_e	H_o/H_e
S1	1.824	0.412	0.235	0.124	0.128	0.969
S2	1.706	0.412	0.294	0.128	0.138	0.928
S3	1.706	0.353	0.353	0.135	0.137	0.985
S4	2.176	0.706	0.353	0.155	0.149	1.040
S5	2.000	0.471	0.294	0.150	0.155	0.968
NP*1	2.167	0.708	0.292	0.124	0.122	1.016

*1: Natural population collected in Tosa Bay. (Taniguchi and Sugama, 1990)
*2: Polymorphic with major alleles less than 0.95.

Table 5. Band sharing indices (BSI) between individuals and mean of BSI within and between groups estimated from DNA fingerprint pattern detected with 33.15 probe and Hinf I restriction endonuclease

No.	No. of bands	Stock 2							Stock 3							
	detected	#36	#35	#34	#33	#8	#7	#5	#36	#35	#34	#13	#12	#11	#6	#5
#36	16															
#35	13	0.207														
#34	15	0.323	0.500			BSI=0.382±0.144							BSI=0.298±0.112			
#33	13	0.138	0.615	0.286												
#8	13	0.414	0.462	0.357	0.231											
#7	14	0.533	0.222	0.276	0.222	0.296										
#5	18	0.412	0.645	0.545	0.516	0.452	0.375									
#36	15	0.194	0.214	0.333	0.071	0.214	0.207	0.242								
#35	16	0.375	0.138	0.258	0.207	0.276	0.200	0.294	0.516							
#34	13	0.414	0.154	0.286	0.308	0.231	0.370	0.129	0.214	0.276			BSI=0.301±0.116			
#13	19	0.286	0.438	0.471	0.500	0.375	0.364	0.486	0.412	0.457	0.375					
#12	12	0.500	0.240	0.296	0.240	0.400	0.385	0.400	0.148	0.214	0.160	0.194				
#11	12	0.286	0.160	0.148	0.240	0.320	0.077	0.333	0.296	0.429	0.240	0.258	0.167			
#6	11	0.296	0.333	0.538	0.333	0.417	0.240	0.345	0.308	0.148	0.250	0.467	0.348	0.348		
#5	15	0.167	0.357	0.467	0.357	0.214	0.167	0.424	0.333	0.258	0.286	0.353	0.444	0.074	0.462	

Table 6. Comparison of mean band sharing indices calculated from the DNA-fingerprints detected by probes 33.15 and YNZ22

	Stock 1	Stock 2	Stock 3	Stock 4	Stock 5	NP
Probe 33.15	0.226	0.382	0.301	0.166	0.206	0.120
Probe YNZ22	0.175	0.279	0.257	0.158	0.122	0.082
Average	0.200	0.331	0.279	0.162	0.164	0.101

Unconscious change in morphological traits

Significant differences were also found in a few morphological traits among the hatchery stocks (Table 7). Since the selection for these morphological traits has not been done systematically, these morphological changes observed in breeding stocks 2-5 may also be caused by unconscious selection.

Escarpment of hatchery stocks and conservation of wild populations

Hatchery stocks have attained genetic changes both in qualitative and quantitative traits which may be caused by conscious and unconscious selections during artificial propagation. On the other hand, the natural populations have been exposed to natural selection for long periods of time, and reached the present stable condition and survival fitness in the natural ecosystem. Ancestors of the hatchery

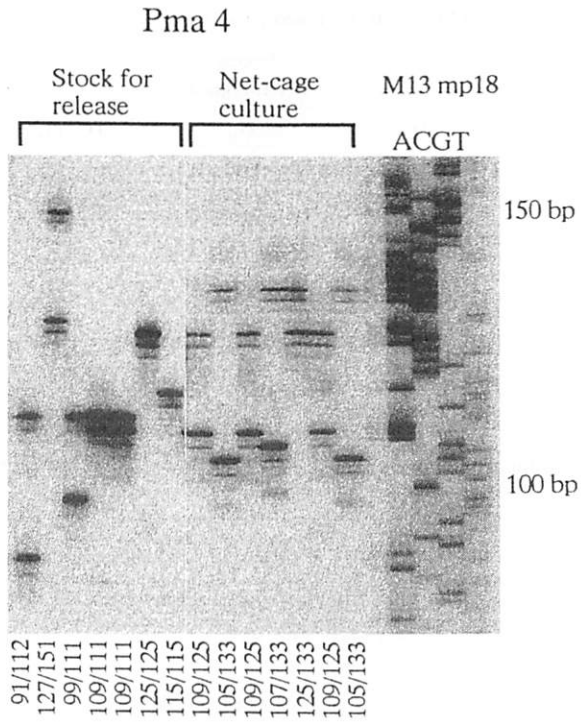

Figure 4. An example of microsatellite DNA pattern of Pma 4 locus. The lanes 1-7 in left are the hatchery stocks 1 for release and the 8-14 the stock 3 for net-cage culture of red sea bream. Right 4 lanes are molecular markers of M13 mp18. Genotypes are shown at the bottom of the figure.

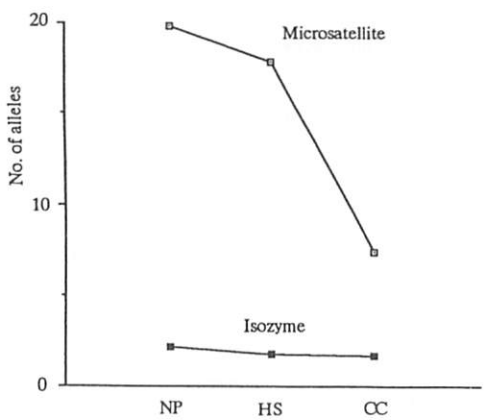

Figure 5. Genetic changes in number of alleles per locus detected from isozyme and microsatellite DNA markers of the hatchery stock 1 for release and the stock 3 for netcage culture compared with those of natural population (NP).

stocks were derived from natural populations and the hatchery population was diversified to a strain for aquaculture, which may have inferior fitness in the natural ecosystem. Since it may be impossible that the hatchery stocks may return to the population having the same level of fitness as the original population, we should find the

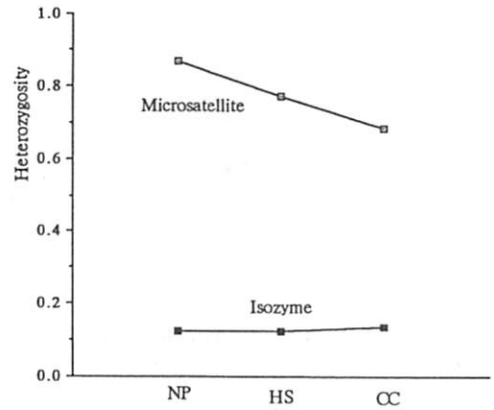

Figure 6. Genetic changes in average heterozygosities detected from isozyme and microsatellite DNA markers of hatchery stocks 1 for release (HS) and stock 3 for net-cage culture (CC) compared with those of natural population (NP).

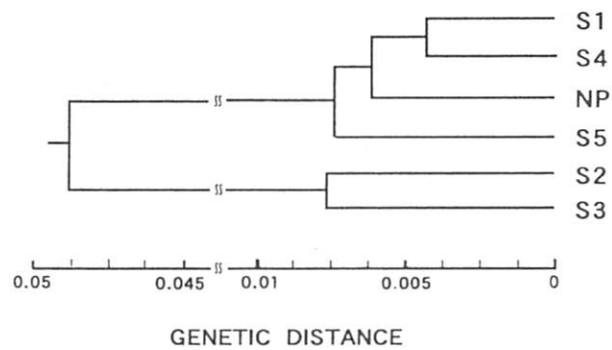

Figure 7. A dendrogram based on the genetic distances between pairs of 5 hatchery stocks and one natural population (NP) of red sea bream.

values on the natural populations as a sustainable genetic resource. We should also be very careful to prevent escarpment of hatchery stocks to the natural water, to conserve the natural population as a genetic resource, because the effects of the escarpment are unpredictable, and not well studied until now.

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Table 7. Comparisons of morphometrical characters (%) of 5 hatchery stocks of red sea bream with statistical analysis[*1]

Characters[*2]	S1 (a)	S2 (b)	S3 (c)	S4 (d)	S5 (e)
BD1/FL[*3]	36.8 ± 0.92^{bcde}	38.2 ± 1.16^{e}	37.9 ± 1.38^{ad}	39.2 ± 1.30^{ccc}	37.9 ± 1.48^{ad}
BD2/FL[*4]	32.9 ± 0.85^{bcde}	33.9 ± 0.91^{ad}	34.1 ± 0.98^{ad}	35.1 ± 1.22^{abce}	34.1 ± 1.23^{ad}
HD/FL	23.3 ± 0.98	23.7 ± 0.99	23.5 ± 0.93	23.7 ± 0.83	23.1 ± 0.60
CPD/FL	9.51 ± 0.37^{b}	9.81 ± 0.24^{a}	9.79 ± 0.28	9.77 ± 0.37	9.77 ± 0.37
HL/FL	26.8 ± 0.54	26.4 ± 0.71^{d}	26.6 ± 0.45	27.0 ± 0.92^{b}	26.5 ± 0.82
OD/FL	7.71 ± 0.47^{bce}	7.10 ± 0.39^{ad}	7.16 ± 0.31^{ad}	7.78 ± 0.30^{bce}	7.12 ± 0.38^{ad}
PAL/FL	55.1 ± 0.87	55.9 ± 1.52^{e}	55.0 ± 0.85	55.6 ± 1.37	54.8 ± 1.47^{b}
SNL/FL	10.9 ± 0.54^{c}	11.3 ± 0.47	11.5 ± 0.47^{a}	11.3 ± 0.61	11.2 ± 0.51

[*1] Strains 1-5 were designated by a-e for ANOVA. Superscripts show significant difference between stocks ($P<0.01$).
[*2] FL: fork length, BD1: body depth 1, BD2: body depth 2, HD: head depth, CPD: caudal peduncle depth, HL: head length, OD: orbital diameter, PAL: pre-anus length, SNL: snout length.
[*3] Body depth measured at 5th spine of dorsal fin.
[*4] body depth measured at last spine dorsal fin.

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THE PRESENT SITUATION AND IMPORTANT ISSUES IN BREEDING BY CHROMOSOMAL SET MANIPULATION IN HIRAME *PARALICHTHYS OLIVACEUS*

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ABSTRACT

The present situation and point at issue in breeding of hirame *Paralichthys olivaceus* using chromosomal set manipulation are reported. The contents discussed here are: (1) production technique of feminized seedlings for commercial culture; and (2) application of chromosomal set manipulation to fixation of traits. In (1), the elucidation of unstable factors which led to the decrease of the proportion of feminization, and the control of sexual maturation in gynogenetic sex reversed female (phenotypically male) are described. In (2), the induction of homozygous fishes in all loci and assessment of characteristics of these fishes, and the induction of cloned fishes and assessment of characteristics of these fishes are described.

BACKGROUND AND OBJECTIVES OF THE STUDY

Since 1984, we have studied the application of chromosomal set manipulation in hirame *Paralichthys olivaceus*[1,2]. During the 7 years from 1984 to 1990, the first developmental period, we studied the basic techniques and problems in producing feminized seedlings, which are dominant in growth than male (Figure 1)[3]. The unstableness of sexual differentiation was recognized as the main problem for the production of feminized seedlings[4,5]. However, since the main cause of this unstableness was clarified as high water temperature at the sexual differentiation period[4,5], the production of feminized seedlings has been realized.

During the following 5 years (1991-1995), the second developmental period, we studied the stability of the production of feminized seedlings[6] and the development of techniques for actual hirame culture in Japan[7]. Simultaneously, basic studies on the application of chromosomal set manipulation for fixation of desirable traits were performed.

The third developmental period started in 1996, in which we are studying methods for the fixation of actual useful traits using chromosomal set manipulation.

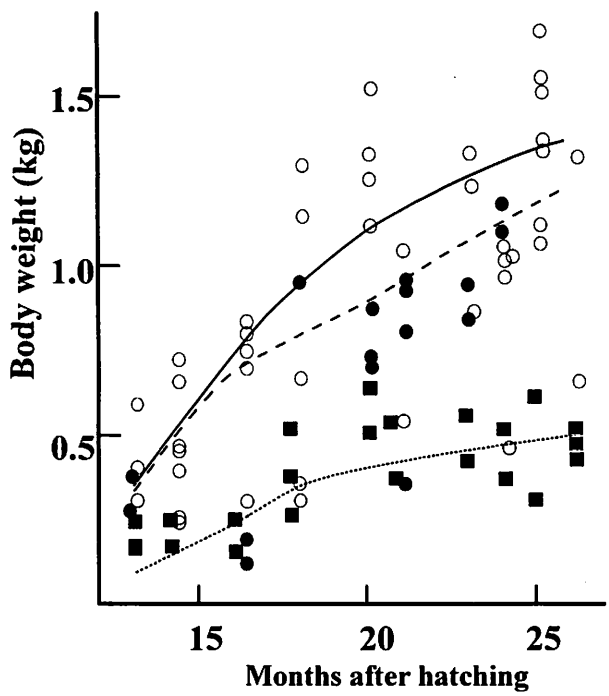

Figure 1. Growth of from 13 to 26 months after hatching in the control diploids (● : female; ■ : male) and feminized seedlings (○ : female; □ : male) of hirame *Paralichthys olivaceus* produced by crossing the sex reversed gynogenetic females (phenotypically males) with the normal females (from Tabata[3]).

PRODUCTION TECHNIQUE OF FEMINIZED SEEDLINGS - FOR COMMERCIAL CULTURE —

Elucidation of unstable factors which led to the decrease of the proportion of feminization

We showed that the main unstable factors were the increase of water temperature and the occurrence of slow growing fishes during the sexual differentiation period[6].

The main sexual differentiation period of hirame is considered to be 20 mm to 50 mm in total length (40th to 70th day after hatching) from the histological study of gonads and the studies on production of gynogenetic sex reversed females[8,9]. Unsuitable factors in the sexual differentiation period are considered to prevent normal sexual differentiation of hirame, because unsuitable factors will stress hirame juveniles and will probably prevent the normal secretion of sexual hormone. As concrete examples of unsuitable factors we confirmed the high temperature and overpopulation from rearing experiments in different water temperatures and rearing experiments of slower growing fish which occurred as result of overpopulation[6]. The results of these experiments are shown in Table 1. Water temperatures in rearing experiments were 18℃ and 23-25℃. The proportion of females in groups reared at 18℃ were distributed around 50% which occurred according to primitive genetic sexual determination. The proportion of females in groups reared at 23-25℃, however, were lower than in groups at 18℃. Further, the proportions of females in slower growing fishes were lower than in the original population, from which slower growing fishes were separated.

If natural hirame larvae hatch out at 14℃ which is the optimum water temperature of spawning, the juveniles will not encounter the water temperature area of 23-25℃ during the sexual differentiation period. Accordingly,

Table 1. The proportion of females in the normal and feminized hirame *Paralichthys olivaceus* groups reared at different water temperatures during sexual differentiation period, and in the slower growing fishes separated from their original group (from Tabata[6])

Group name	Rearing water temp. (℃) 0-20	25-45	50+ (days after hatching)	Sample size	Proportion of females (%)	Density (number/m^2) *1
Normal diploid						
N1-18	18	18	18	65	43.1	1,140
N1-18-L	18	18	18	30	20.0	204
N1-23	20	23	25	33	24.2	1,572
N1-23-L	20	23	25	28	0	532
N2-18	18	18	18	24	50.0	968
N2-18-L	18	18	18	21	9.5	244
N2-23	20	23	25	35	14.3	424
N3-18	18	18	18	37	54.1	216
N3-23	20	23	25	34	26.5	804
N4-18	17	18	18	30	43.3	314
Feminized seedling						
F1-18	17	18	18	51	80.4	897
F1-18-1 *2	17	18	18		88.3	
F1-18-2 *3	17	18	18	32	56.3	
F2-18	17	18	18	30	90.0	722
F1, F2-18-L	17	18	18	30	23.2	

N and F in group name indicate normal deploid and feminized seedling production, respectively.
Numbers following N and F indicate the experimental number.
-18 or -23 in group name indicates rearing temperature during 25-45th day after hatching.
-L in group name indicates lower growing fishes selected from the original group.
*1 : At 53-55th day after hatching in 18℃ rearing, and at 43-44th day after hatching in 23℃ rearing.
*2: Group of small size fishes (F1-18-2) from F1-18 at 120th day after hatching.
*3: Group of small size fishes selected from F1-18 at 120th day after hatching.

hirame larvae hatched at the optimum water temperature for spawning will express the genetically determined sex

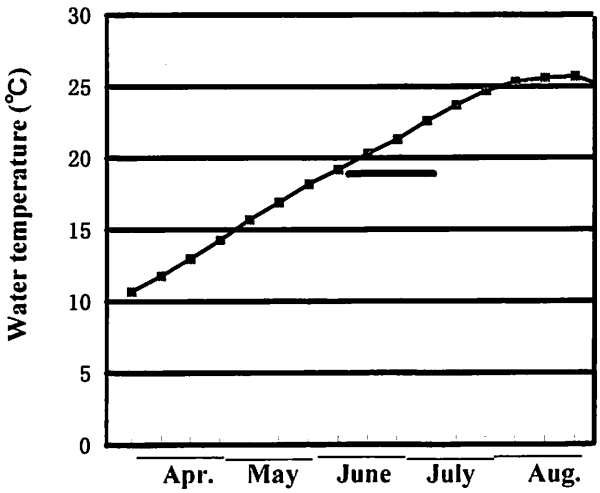

Figure 2. Progress of the natural seawater temperature (curve) in Akashi, Hyogo Prefecture and the estimated sexual differentiation period (line) of natural hirame *Paralichthys olivaceus* larvae hatched out at 14℃ which is the optimum water temperature of the spawning.

(Figure 2)[10].

The slower growing fishes often appeared during the period when the average total length in the original population reached about 20 mm. By then, hirame larvae have finished metamorphosis and begin to settle on the bottom. When hirame juveniles were able to inhabit the bottom and eat freely, their body color changed to clear. However, in cases of overpopulation, the slower growing fish had to swim continuously at the surface, and were unable to move to their demersal habitat. These slower growing fish showed a darker body color while swimming, but when they were transferred into a new tank, they immediately inhabited the bottom. They soon began to take food, and their body color became clear. They grew favorably and reached almost the same size as the fish in the original population by the 100th day after hatching.

From the results mentioned above, it is important to avoid high water temperature and overpopulation during the sexual differentiation period to obtain stable and high proportion of females in feminized seedling.

Control of sexual maturation in gynogenetic sex reversed female (phenotypically male) ('pseudo-male')

Early seedlings are required for the realization of high growth and survival. We clarified a stable method to

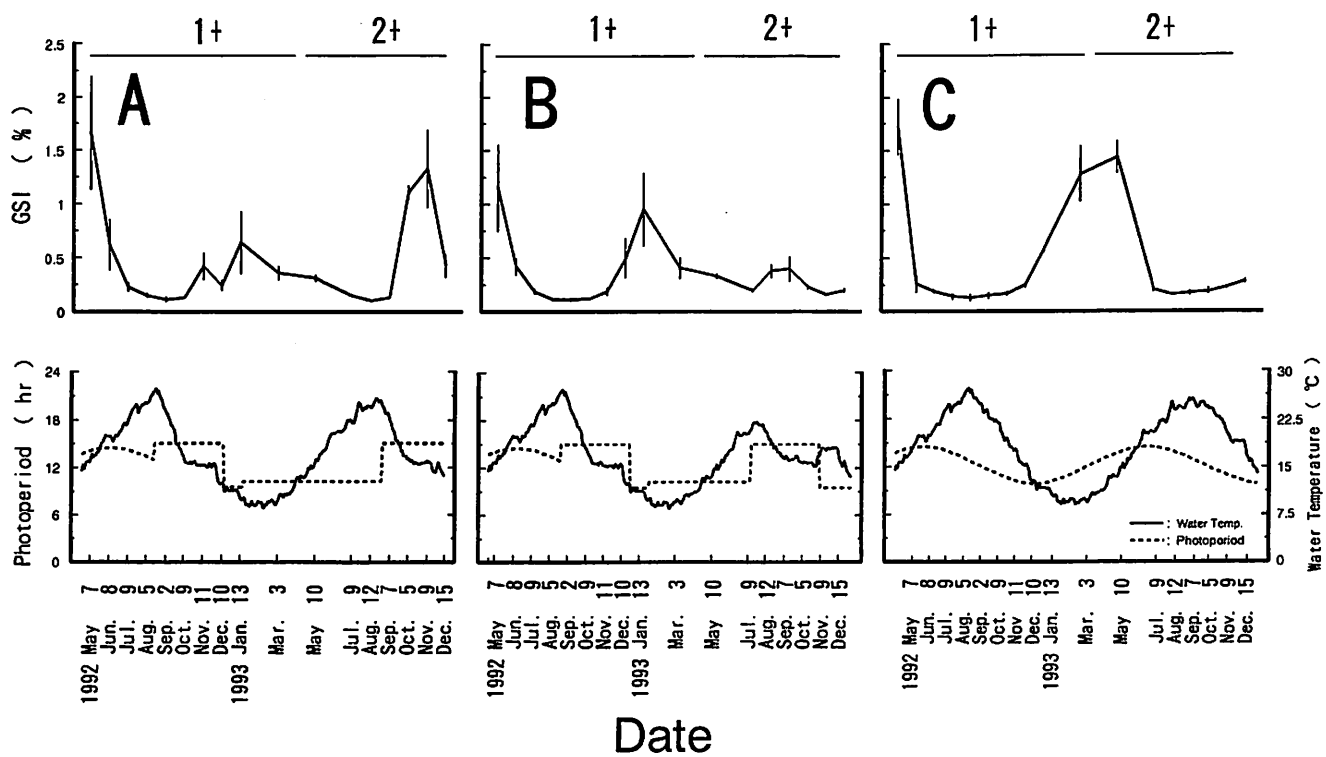

Figure 3. Change of gonad somatic indices (GSI) in the sex reversed gynogenetic females (phenotypically males) hirame *Paralichthys olivaceus* reared under the different photoperiod and water temperature (group A and B). C (control) was reared under the natural photoperiod and water temperature. Each bar shows the mean +/- SD (from Mizuta et al.[7]).

accelerate the sexual maturation by the progress of gonad-somatic index (GSI) and the histological observations of the gonads of pseudo-males reared under a controlled environment[7].

In order to investigate the condition of sexual maturation of pseudo-males due to a long photoperiod and low water temperature treatments, two groups were reared under the following different conditions. One group was reared under a photoperiod twice as long and low water temperature treatments with an 8 month short photoperiod during these treatments (group A). The other group was reared under a photoperiod twice as long and low water temperature treatments with a 6 month short photoperiod during these treatments (group B). The control group was reared under both natural temperature and photoperiod. Their testicular maturation was assessed from GSI and histological section.

Although GSI increased due to a long photoperiod and low water temperature treatments, the testicular maturation was observed only in group A after a second treatment (Figure 3). Further, it was observed that cysts in group A had been filled with spermatogonia (Figure 4), but cysts in group B had not been filled before the second treatment. From these results, it was suggested that at least an 8 month short photoperiod before the next treatment had to be taken for the acceleration of testicular maturation of pseudo-males due to long photoperiod and low water temperature treatments.

Production of feminized seedling by an aquaculturist

Since 1994, 100,000 - 150,000 feminized seedlings for pond culture a year have been produced by an aquaculturist in Hyogo Prefecture.

APPLICATION OF CHROMOSOMAL SET MANIPULATION TO FIXATION OF TRAITS

Induction of homozygous fish and assessment of characteristics of these fish

The first step for cloned fish production is to induce the homozygous fishes of gynogenetic diploids by the suppression of mitosis. Various examinations to produce stable homozygous fishes and to assess their characteristics were performed.

In order to know whether hirame induced by the suppression of mitosis is homozygous, we investigated isocitric acid dehydroginase (Idh) allele of hirame induced. When Idh allele of the female parent used is heterozygous, this allele in gynogenesis induced by the inhibiting meiosis was all heterozygous[2,11], but in gynogenesis induced by the suppression of mitosis it was all homozygous[12]. This phenomenon is considered to be caused by gene-centromere recombination which occurs by crossover of chromosomes, i. e., the heterozygous rate in gynogenesis

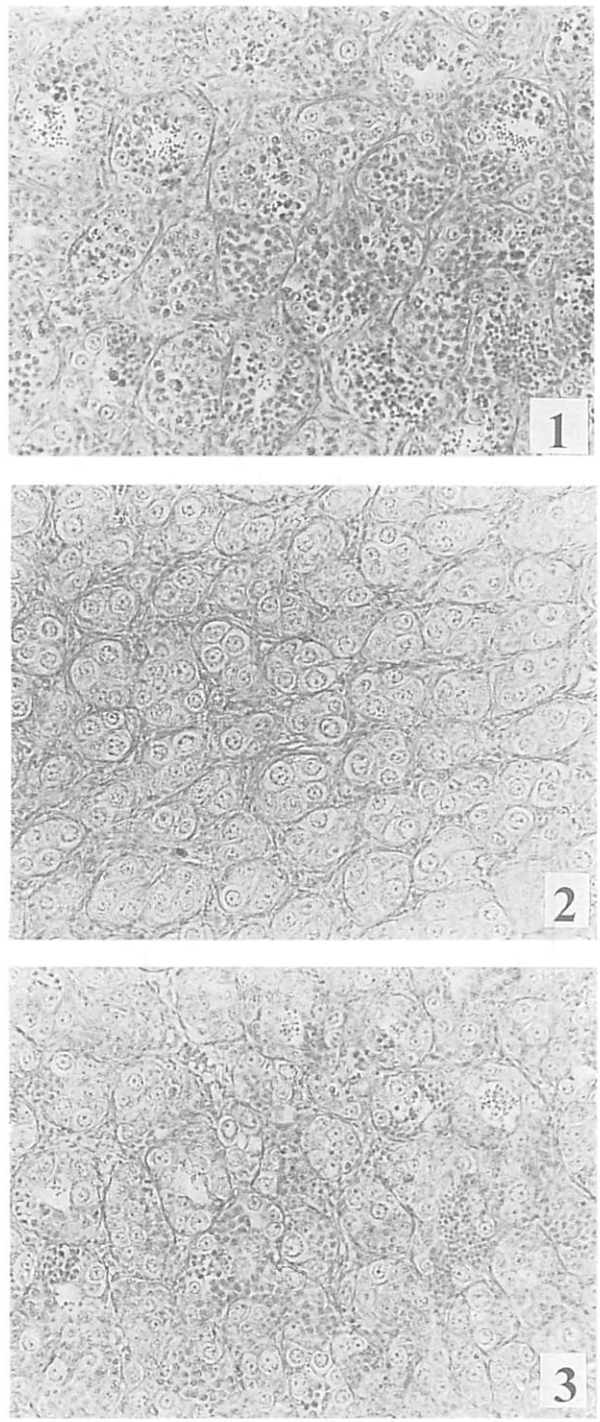

Figure 4. Histological sections of testes in the 2+ sex reversed gynogenetic females (phenotypically males) hirame *Paralichthys olivaceus* (group A) (from Mizuta et al.[7]).
1: Testis at 2 months before the start of the acceleration treatments; 2: Testis at 1 month before the start of the acceleration treatments; 3: Testis at the start of the acceleration treatments.

induced by inhibiting meiosis is high as when the locus situates far from the centromere, on the contrary, the heterozygous rate is low as when the locus situates near to the centromere[13]. When the heterozygous rate in Idh allele of gynogenetic hirame induced by inhibiting meiosis is 100%, Idh locus in hirame is considered to situate far from the centromere[11]. Accordingly, if Idh allele are homozygous, it will mean success of the induction of gynogenesis by the suppression of mitosis.

Figure 5 shows Idh isozyme in both gynogenesis induced by the inhibiting meiosis and gynogenesis induced by the suppression of mitosis using female parents in which Idh alleles are heterozygous. As mentioned above all Idh isozymes in gynogenesis induced by the inhibiting meiosis are heterozygous and all Idh isozymes in gynogenesis induced by the suppression of mitosis are homozygous.

The hatching rate and normal hatching rate (to used egg) of gynogenetic diploids induced by the suppression of mitosis are shown with hatching rate of control diploids in Figure 6. The hatching rates of the gynogenetic diploids were lower than those in control diploids. Although the normal hatching rate was by far lower than the hatching rate, the mean normal hatching rate was stable at about 4%. In spite of the low rate, 4,000 homozygous hatched fry will be given from one gynogenetic treatment as 100,000 stripping eggs can be stripped from a female parent.

In homozygous diploids the larger divergence will be expected because heterozygous loci are divided. The phenomenon was confirmed by DNA fingerprint. The example is shown in Figure 7. From these DNA fingerprints, band-shared indices (BSI) are calculated (Table 2). The BSI values of homozygous diploids were lower than control diploids although higher than natural population which will have diversity[14].

The proportion of females and the proportion with gonadal abnormalities in the homozygous diploids are shown in Table 3. The proportion of females seems to be divided into high and low groups, i. e., the high proportion groups with approximately 90%, and the low proportion groups with approximately 50%, respectively. As mentioned later, the proportion of females in clones induced from these each group gave interesting results. The high proportion with gonadal abnormalities in the homozygous diploids is also noticed. The abnormal pattern was various, i.e. either of two gonads is abnormal but other is normal; both gonads are normal or abnormal. However, the pattern that either gonad is abnormal tended to be many. Tsubaki[15] reported that such a fluctuating asymmetry may be caused by genetic stress as the decrease of heterozygosity. The unzygomorphism of gonads in homozygous diploid hirame observed in our experiments may be also caused by homozygosity. These females will be used for breeding except for those females with both abnormal gonads.

Figure 5. IDH isozyme patterns of hirame *Paralichthys olivaceus* (from Tabata and Gorie[12]).
A: Control diploids. IDH genotypes of the parents were *AB* (female) X *AB* (male).
B: Gynogenetic diploids induced by the inhibition of the 2 nd meiosis. IDH genotype of the female parent was *AB*.
C: Gynogenetic diploids induced by the suppression of the 1 st cleavage. IDH genotype of the female parent was *AB*.

Induction of cloned fish

Two types of cloned fish (homotype and heterozygous clone) were gained, and the characteristics in cloned fishes were assessed.

The induction of cloned fish was confirmed by DNA fingerprinting using minisateraite as a probe. The DNA fingerprints are shown in Figure 8. The homotype clones had 16 distinguishable fragments which were entirely shared with their mother. The heterotype clones had distinguishable 30 fragments, in which 9 fragments were shared with only their mother, 7 fragments were shared with only their father, and 7 fragments were shared with both their mother and father.

The hatching rate, the proportion of females, the proportion with body color abnormalities, the proportion of malformations, and the proportion with eyes on right side of body in cloned fishes are shown in Table 4.

The hatching rates in homotype clones were lower than in control diploids although there were examples that the former was higher than or the same as the latter. These phenomenon may be caused by homozygosity. On the contrary the hatching rate in heterozygous clones were the

Table 2. Band-sharing indices (BSI) among gynogenetic diploids induced by the suppression of mitosis with BSI among control diploids and among natural hirame (from Tabata and Adachi[14])

	BSI average	SD
Control diploids	0.705	0.111
Mitotic gynogenetic diploids *	0.596	0.226
	0.628	0.208
Natural hirame population	0.307	0.132

* Gynogenetic dipoloids induced by the suppression of mitosis
BSI = 2 $(N_{a+b})/(Na+Nb)$
Where
N_{a+b}: Number of shared fragments
Na: Number of fragments in individual A
Nb: Number of fragments in individual B

Table 3. The proportion of females and the proportion with gonadal abnormalities in gynogenetic homozygous diploids induced by the suppression of mitosis

Group	No. investigated	Proportion with gonadal abnormalities (%)		Proportion of females (%)
		One side	Both sides	
91B9	116	18.1	19.8	89.5
91B3	48	8.3	31.3	51.4
91B1	27	-	-	48.1
92B1,2,4	7	0	0	85.7
92B3	7	14.3	0	85.7
92B6	59	35.6	10.2	55.9
92B7	57	33.3	1.8	94.7
92B9	35	48.6	0	37.1

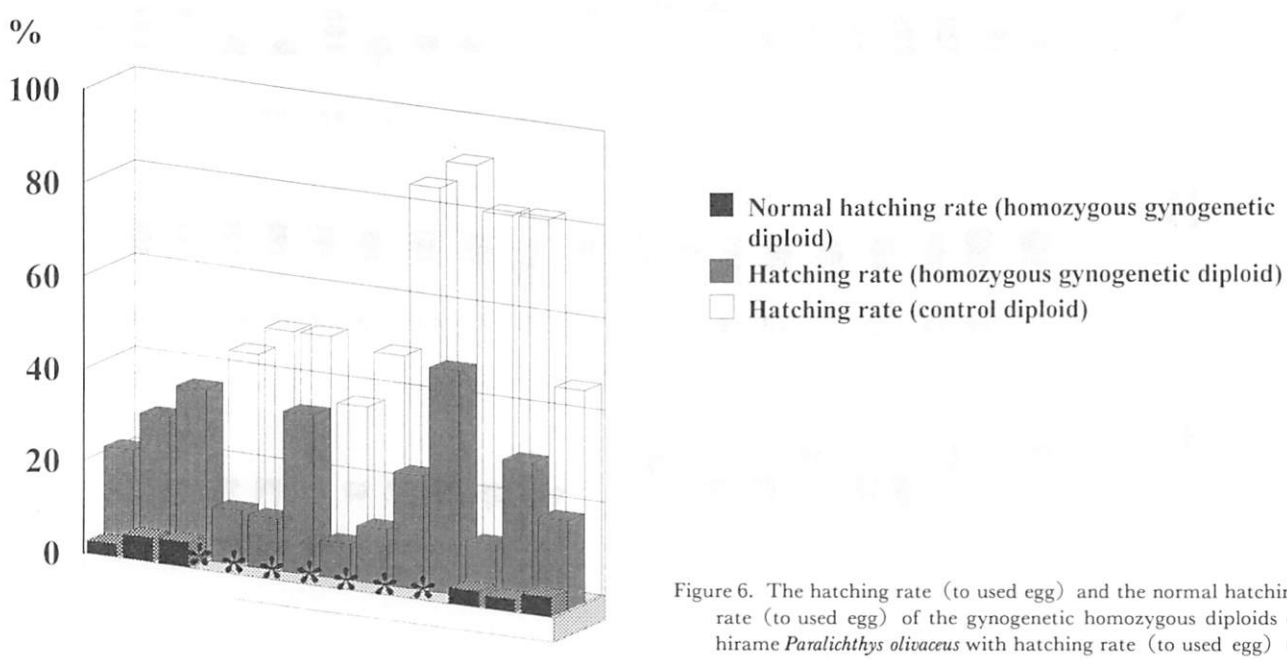

Figure 6. The hatching rate (to used egg) and the normal hatching rate (to used egg) of the gynogenetic homozygous diploids of hirame *Paralichthys olivaceus* with hatching rate (to used egg) of the control diploids.
* Normal hatching rates were not measured, but approximated to the other measured rates.

same as in the control diploids, and there were no extremely lower examples than the control diploids.

The proportion of females in homotype clones was high; however, the proportion in group 95C5 decreased a little. The cause of the decrease is considered to be the occurrence of the slower growing fishes by overpopulation because the proportion of females of slower growing fishes (95C5-L) in group 95C5 was as low as 16%. It was shown that slower growing fish also occur in clones when the rearing density was high. On the contrary the proportion

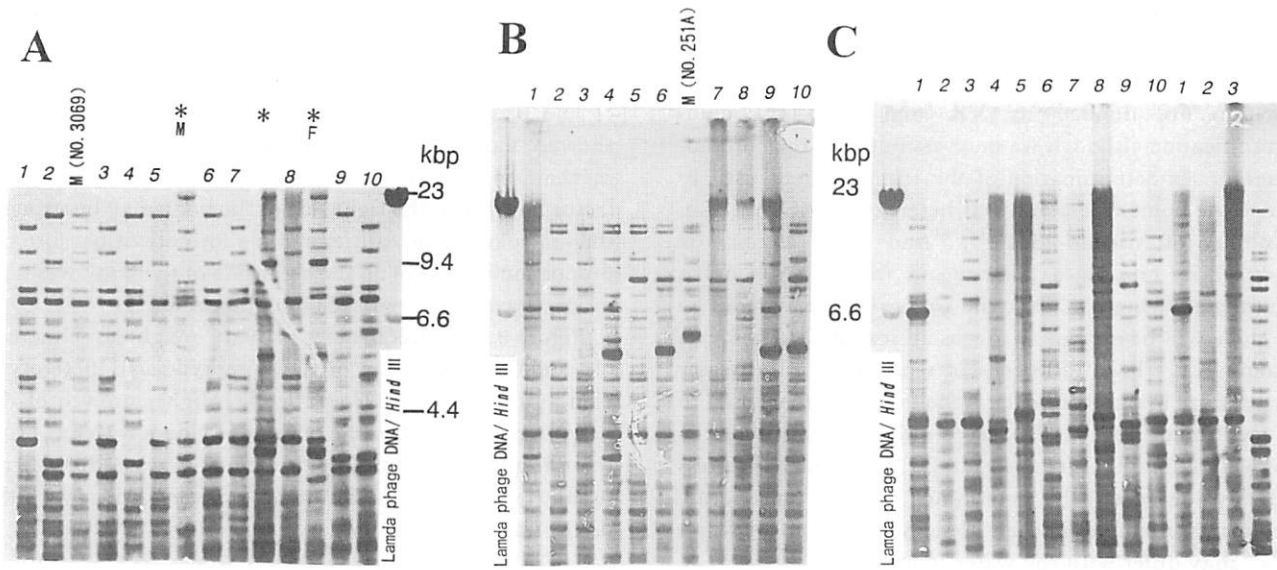

Figure 7. DNA fingerprints of gynogenetic diploids (A, B) of hirame *Paralichthys olivaceus* induced by the suppression of mitosis with the control diploids (*) and natural caught hirame (C).
M and F indicate the mother and father, respectively (from Tabata and Adachi[14]).

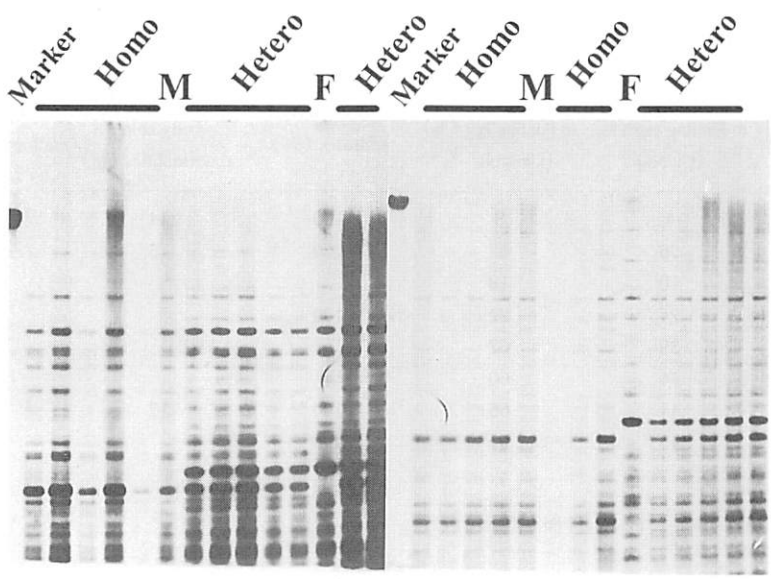

Figure 8. DNA fingerprints of the homotype clones produced from the gynogenetic homozygous diploids of hirame *Paralichthys olivaceus*, and of the heterotype clones produced by crossing between the father and mother of the gynogenetic homozygous diploids.
M and F indicate the mother and father, respectively.

of females in heterotype clones was extremely low. Male parents used for induction of heterotype clones were picked from the homozygous group in which the proportion of females were distributed around 50%, except that female parents were picked from the homozygous fish group in which the proportion of females was distributed around 90%. As to the causes of the low proportion of females in the heterotype clones, we propose the following two hypotheses. One: chromosomes (X, Y) for sex determination are divided to XX and YY by homozygositification although we must assume that the chromosomes for sex determination of the female parent used to induce the homozygous fish was heterozygous (XY) (i.e. possibility of 'pseudo-female'). And so, if a clone is produced by crossing a homozygous male (YY) and a homozygous female (XX), the proportion of females in the clone will be extremely low as all sexual chromosomes become XY. Two: difference of sensitivity to the water temperature may be present. If the female parents used to induce the homozygous fish have different sensitivities to the water temperature (e.g. 18-20 ℃) which express correctly the genetically determined sex of a group, the proportion of females in each homozygous group (90 and 50%) may differ with the water temperature.

The proportion with body color abnormalities and the proportion of malformations in the homotype clones are higher than in heterotype clones as shown in Table 4. These abnormalities are reported to be depended on vitamin A level for larval hirame[16,17]. However, the genetic factor on malformation was also indicated as the difference in the sensitivity factor in the study of red sea bream[18]. If we apply the sensitivity factor to our results, it is suggested that the sensitivity factors on body color abnormalities and malformations are easy to appear by the homozygositification in homotype clones. On the contrary, these sensibility factors were masked by the heterozygositification in heterotype clones.

The proportion with eyes on the right side of body showed various results. Although basically the same mechanism as mentioned above will act, the sensitivity factor for eyes on the right side of the body may be stronger than in color abnormalities and malformations because the proportion with eyes on the right side of the body was high or low extremely even in homotype clones.

Figure 9 show frequencies of body weight at 7 months old and 19 months old of the homotype and heterotype clones produced in 1993 and 1994. Higher growth of the heterotype clones than the homotype clones is clear. From these results, the future production of clone seedlings with useful traits may be performed by crossing clones with the useful traits.

The future breeding using cloned fish

Study on cloned fish production with the useful traits has been started in 1996. We are examining clones with

Table 4. The hatching rate, the proportion of females, the proportion with body color abnormalities, the proportion of malformations, and the proportion with eyes on right side of body in the homotype and heterotype clones

	Group	Hatching rate to floating egg (%) (Clones)	Hatching rate to floating egg (%) (Controls) *	Proportion of females (%)	Proportion with body color abnormalities (%)	Proportion of malformations (%)	Proportion with eyes on right side of body (%)
Homotype clones	93C4	4	9	100	14	19	34
	93C7	56	52	100	16	28	0
	94C5	10	18				
	94C7	24	22	100			
	94C9	39	62				
	94C123	74	60				
	94C15	44	66	100	12		0
	95C5	45	91	88			
	95C5-L			16			
	94C6	9	59				
	94C8	23	65				
Heterotype clones	93C12	39	42	0	0	0	0
	93C12-L			0	0	3	7
	94C112	49	60	5	1		2
	94C14	83	66				

-L in group name indicates lower growing fishes selected from the original group.
*: Inseminated sperm of normal diploid to the same eggs used to induce clones.

disease resistance. Maintaining cloned strains will be performed by self-breeding of homotype clones. However, the actual production of seedlings with useful trait (s) will be performed by crossing cloned strains. DNA markers related with special traits will be studied further.

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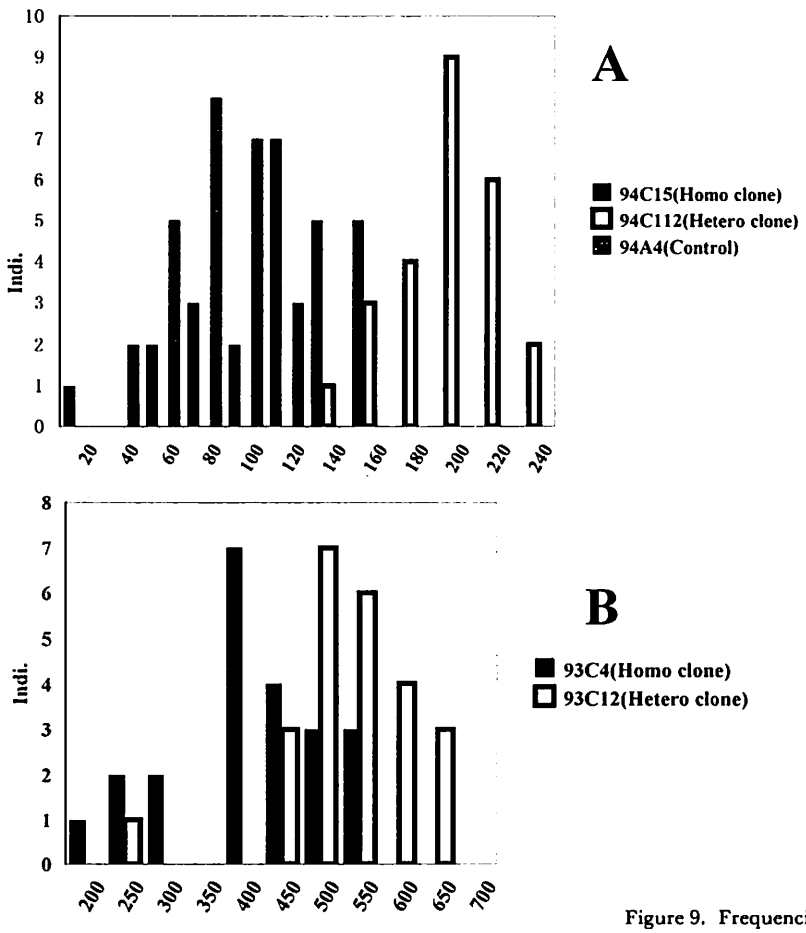

Figure 9. Frequencies of the body weight at 7th month after hatching of homotype and the heterotype clones of hirame *Paralichthys olivaceus* produced in 1994 (A), and 19th month after hatching of the homotype and heterotype clones of hirame produced in 1993 (B).

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INTRODUCTION OF NONINDIGENOUS SPECIES FOR AQUACULTURE IN JAPAN

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ABSTRACT

Summarized is a history and the state of introduction of nonindigenous species for aquaculture in Japan. Presented as an example is the result of introduction of sturgeon introduced by the exchange project of fish fry between Japan and the USSR (present Russian Federation). Rainbow trout *Oncorhynchus mykiss* was introduced from the United States in 1877. Since then, more than 70 species have been introduced from over 20 countries for aquaculture. Many of these fish, however, failed to settle in Japan, with the notable exceptions of rainbow trout, European eel *Anguilla anguilla*, tilapias *Oreochromis* sp., and several other species. In recent years, sturgeon culture has become a focus for aquaculture, however, difficulty in reproduction has been an obstacle to successful culture. Due to the success of the authors' group in the species' reproduction in 1988, many people have become interested in sturgeon culture. These past examples teach us the importance of investigations before introduction and examinations after introduction about the taste (fitness for Japanese taste); breeding (fitness for Japanese environment, technique and facilities); supply of fry (stable import or reproduction domestically); diseases; and economic value of the fish.

INTRODUCTION

In the field of aquaculture, many kinds of nonindigenous fish have been introduced to meet consumer demand and to develop the aquaculture industry. The oldest fish introduced in Japan is said to be the goldfish *Carassius auratus*, an ornamental fish, from China in 1502. As a food fish, the first introduction was rainbow trout *Oncorhynchus mykiss* from the United States in 1877. Since then, more than 70 species of finfish have been introduced, but the production of only five species are recorded on the fishery statistics. Many others failed to settle in Japan as food fish.

In this paper, we chose the fish which were introduced for aquaculture from our previous report[1)] and summarized the beginnings of the introductions and the present status of the fish.[2,3)] The objectives of this paper are to learn ways of successful introductions of nonindigenous species for aquaculture from the past successful and unsuccessful examples.

SUCCESSFUL INSTANCES

The productions of the following five species, rainbow trout, European eel *Anguilla anguilla*, tilapias (*Oreochromis niloticus* and *O. aurea*), and coho salmon *Oncorhynchus kisutch* are mentioned in the nationwide fishery statistics. These fishes were regarded as successful instances of introduction here.

Rainbow trout

Ten thousand fertilized eggs of rainbow trout were imported from California in 1877 for the first time. Since then, it has been introduced several times from the United States, and many were implanted into several lakes and rivers before rainbow trout culture became popular. Accordingly, rainbow trout catch became the most among prevalent landlocked salmon in the 1970s and popular as an object of sport fishing. Rainbow trout culture has prospered since 1951 when the export of frozen trout to the United States was begun. But the export was decreased sharply by a high yen rate after the 1980s, and almost all of the products have been supplied for domestic consumption in recent years. After 1982 when its production reached maximum at 18,230 ton, it has been decreasing gradually at 14,364 ton in 1993 (Figure 1 A).

Tilapias

Since the first tilapia, *Oreochromis mossambicus*, was introduced in Japan from Thailand in 1954, 10 species of

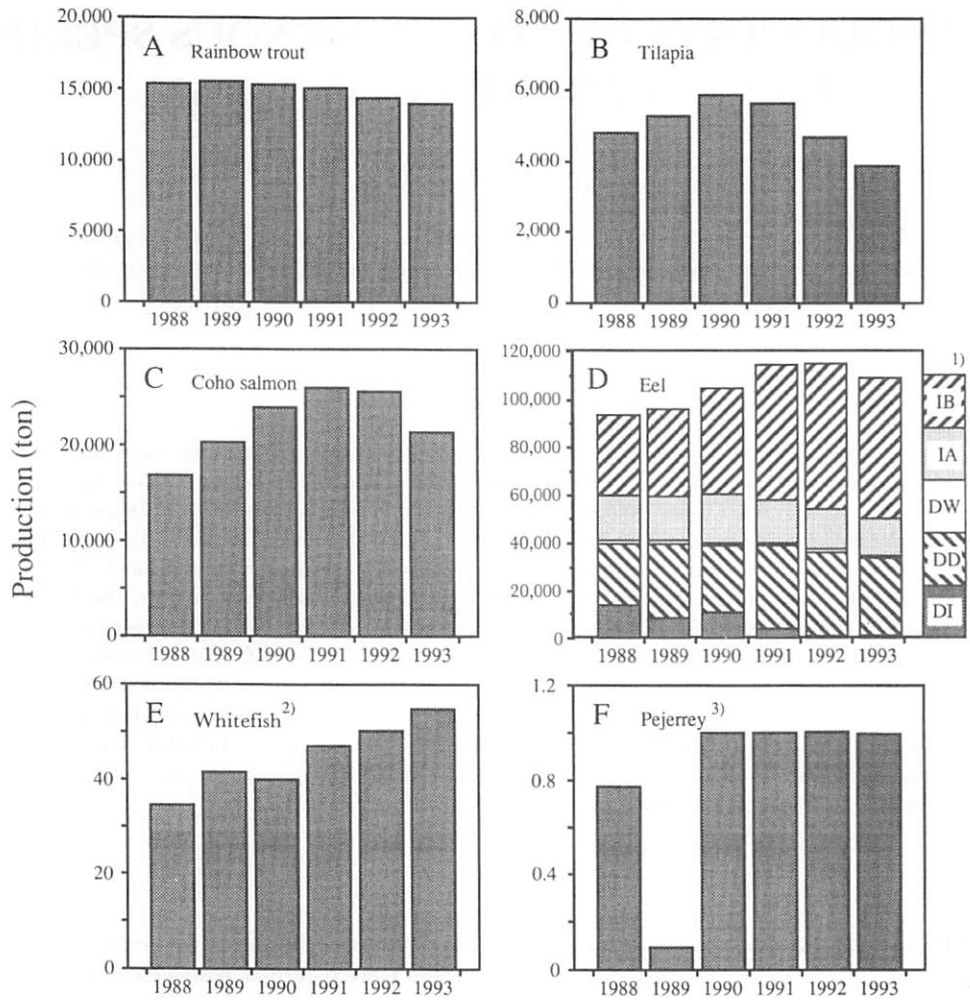

Figure 1. Nonindigenous fish production by major species/groups, 1988-93.
1) IB, imported baked eel; IA, imported live eel (commercial size); DW, domestic wild eel; DD, domestic cultured eel originated from domestic fry; DI, domestic cultured eel originated from imported fry (estimated value).
2) Production in Nagano Prefecture.
3) Production in Kanagawa Prefecture.

cichlid fish have been introduced. The places of origin are Africa and the Middle and Near East, but half of them came to Japan by way of other places. *O. sparrmanii* in 1959, *O. macrocephala* in 1960, *O. galilaea* in 1964, and *O. macrochir* in 1964 were introduced from the United States and *O. aurea* from Taiwan in 1980. Nile tilapia *O. niloticus* which is the main species for aquaculture in Japan was introduced from Egypt in 1962 together with *O. zillii*. Another two are *O. butikoferi* from Sierra Leone in 1980 and *O. hornorum* from Israel in 1981. Tilapia was popular because of its white meat and simple taste, similar to that of red sea bream *Pagrus major*, which is regarded as "king of the fish" in Japan, and its production has been developed since 1976 when it appeared in fishery statistics for the first time. But after 1990 when its production reached a maximum at 5,825 ton, the production has been decreasing (Figure 1 B). The reasons of the decrease are guessed to be caused by defectiveness of the nationwide marketing system of the products and depreciation of the price influenced by depreciation of the price of red sea bream. In recent years, hybrid tilapia between *O. niloticus* females and *O. aurea* males has been cultured to raise male ratios for efficient production. *O. mossambicus* and *O. zillii* are not cultivated commercially now, but settle in natural waters in the southern part of Japan.

Coho salmon

Coho salmon is mentioned here though it is a indigenous species, for its culture in Japan has depended on fertilized eggs imported mainly from Washington and Oregon since 1965. Its production in 1993 was 21,148 ton which was the fourth major production in Japan's aquaculture following those of yellowtail *Seriola quinqueradiata*, red sea bream, and

eel. This industry had grown smoothly before several years ago (reached peak at 25,730 ton in 1991), but its production has been decreasing gradually because of depreciation of the price caused by excessive production and competition with imports from Chile (Figure 1 C). Many farmers are making efforts to produce fish of better quality and to reproduce them domestically for more efficient culture.

Eels

Eel culture in Japan was begun in 1879. The production of eel had been increased year after year and accounted for more than half of the total world eel production at the end of the 1960s. The increase caused a serious lack of eel fry, thus eel fry from 13 countries has been introduced for filling in the lack so far. The first introduction of non-indigenous eel was European eel from France in 1969. Since then, several species, *A. dieffenbachi* and *A. schmidti* from New Zealand in 1970, *A. rostrata* from the United States in 1971, and *A. bicolar pacifica* from Philippine in 1972, were introduced. But these species have not been popular because of differences in Japanese eel *A. japonica* in appearances, tastes, and breeding techniques. Even the import of European eel fry which is the most popular species of nonindigenous eel have decreased from 5,170 kg in 1988 to 865 kg in 1993. Japanese eel had also been introduced from Korea, Hong Kong, China, Taiwan, and North Korea since 1964. But in recent years, these countries have prohibited export of eel fry because they were securing the fry for their own farmers. The introduction of eel fry has decreased sharply. On the other hand, the amount of import of commercial size eel and baked eel from China and Taiwan has increased remarkably and reached twice that of domestic production (Figure 1 D).

UNSUCCESSFUL INSTANCES

The fishes included here as unsuccessful instances are not cultivated as food fish now. Some died out, some still survive in natural waters, and some are reared in a few limited places.

Died out

Crappie *Pomoxis nigromaclatus* from Oregon in 1927, striped bass *Roccus saxatilis* from California in 1927, American shad *Alosa sapidissima* from Oregon in 1928, yellow perch *Perca flavescens*, yellow bass *Morone interrupta*, and freshwater drum *Aplodinotus grunniens* from Illinois in 1960, four species of carp (*Catla catla*, *Barbus tor*, *Labeo rohita*, and *Cirrhina mrigala*) from India in 1960, golden shiner *Notemiginus chrysoleucus* from California in 1967, and swamp eel *Fluta alba* from Hong Kong in 1968 have died out from accidents, diseases, or maladjustment to Japanese environments.

Survivors in natural waters

Grass carp *Ctenopharygodon idellus*, silver bighead *Hypophthalmichthys molitrix*, striped bighead *Aristichthys nobilis*, and black Chinese roach *Mylopharyngodon piceus* from China in 1878, brook trout *Salvelinus fontinalis* from Colorado in 1901, snakehead mullet *Channa maculata* from Taiwan in 1906, brown trout *Salmo trutta* from the United States, crucian carp *Macropodus chinensis* from North Korea in 1914, mosquitofish *Gambusia atfinis* from Taiwan in 1916, northern snakehead *Channa argus* from North Korea in 1923, largemouth bass *Micropterus salmoides* from the United States in 1925, rose bitterling *Rhodeus ocellatus* from China in 1942, bluegill *Lepomis macrochirus* from Chicago in 1960, and tench *Tinca tinca* from Holland in 1961 are not cultivated commercially as food fish but have survived in natural waters. Some species such as the largemouth bass and bluegill are popular for sport fishing.

Others

Rudd *Scardinus erythrophalmus* from Britain in 1963, lake trout *Salvelinus namaycush* from Canada in 1966, and giant gourami *Osphronemus goramy* from Thailand in 1981 are reared in a few limited places. Rudd is used for freshwater pearl culture as a host for larvae of the pearly freshwater mussel *Hyriopsis schlegeli*. Lake trout is reproduced in the Nikko branch of NRIA (the National Research Institute of Aquaculture) but prohibited to be spread for fear of disturbing the ecosystem because of its fierce carnivorousness. And silver bighead is cultured to clean Lake Kasumigaura, Ibaragi Prefecture, and to make use of its pituitary for artificial propagation of fish.

Several species were regarded as unsuccessful instances for the present though all of them have not been concluded to be unsuitable for aquaculture in Japan. There is a possibility that some of them may be promoted for development or successful introductions in the future.

DEVELOPING INSTANCES

As examples of hopeful object of aquaculture in the future, whitefish *Coregonus* sp., pejerrey *Odonthestes bonariensis*, sturgeon, chinook salmon *Oncorhynchus tschawytscha*, and channel catfish *Ictalurus punctatus* should be enumerated.

Whitefish

The first introduction of whitefish was 2,700,000 fertilized eggs of *Coregonus clupeaformis* from Ohio in 1926. After that, *C. olbus* from Ohio in 1926, *C. lavaretus baeri* in 1929, *C. lavaretus maraena* in 1929, *C. autumnalis migratorius* in

1969, *C. lavaretus ludoga* in 1981, and *C. muksun* in 1981 from the USSR, and *C. peled* from Czechoslovakia in 1972 had been introduced. The first four species introduced in the 1920s were implanted into some lakes but did not settle there. The main species of whitefish culture in Japan is *C. peled* which originated from Czechoslovakia and the USSR during the period 1975-1983. Its product is still not so large but has been increasing year by year (Figure 1 E).

Pejerrey (Argentine silversides)

Seventy thousands of pejerrey eggs were introduced from Argentina in 1966 for the first time. Pejerrey has a good reputation for its plain white flesh. Its production, however, has not increased because of the pejerrey's slow growth and weakness in handling (Figure 1 F). Accordingly, 10 prefectural research institutes organized a study group of pejerrey culture, and pejerrey production is expected to be developed in the future.

Sturgeon

Sturgeon culture is the newest industry of fish culture in Japan. The main fish of sturgeon culture is bester (hybrid between *Huso huso* female and *Acipenser ruthenus* male) as mentioned later. The first sturgeon introduced as experimental fish for aquaculture were 41 fry of Russian sturgeon *A. giildenstadti* from the USSR in 1963, but all died within 1 week after arrival. In the next year, 474 fly of Siberian sturgeon *A. baeri* were sent from the USSR. These fish were distributed to 9 organizations, and 154 out of 474 fry were bred and used for examinations in the Freshwater Fisheries Research Laboratory (presently NRIA). The last Siberian sturgeon of NRIA died during the study of artificial spawning in 1985. The first white sturgeon *A. transmontunus* was transported to Suma Aquarium in Kobe from Washington in 1961. Since then several aquariums imported the fish from Canada and the United States. The first white sturgeon for aquaculture was imported by Sunrock Co., Ltd. in Miyagi Prefecture from California in 1989. Sunrock succeeded in reproducing white sturgeon using 8-yr.-old fish obtained from an aquarium in 1992 and is planning to supply fry to fish farmers in the near future.

Chinook salmon

The first introduction of chinook salmon was from the United States in 1881, but details about that are not clear. During the period 1959-1968, about 2,500,000 fertilized eggs of chinook salmon were introduced and released after hatching into the River Tokachi and the River Teshio in Hokkaido but had not returned there. On the other hand, cage culture of chinook salmon was begun in Niigata Prefecture in 1983, but information about the actual situation of chinook salmon culture could not be obtained.

Channel catfish

Since 1971, channel catfish has been introduced several times from California and Alabama. It is expected to be the substitute for Japanese catfish which is hard to culture because carnivorousness. But the information about channel catfish production in Japan could not be obtained.

EXCHANGE PROJECT OF FISH FRY BETWEEN JAPAN AND THE USSR

The exchange project of fish fry between Japan and the USSR through a cooperative agreement in fisheries science and technology was carried out between 1968 and 1988. As shown in Table 1, bester, Atlantic salmon *Salmo salar*, coho salmon, kokanee *Oncorhynchus nerka*, and four species of whitefish (*Coregonus autumnalis migratorius*, *C. peled*, *C. lavaretus ladoga*, and *C. muksun*) were introduced in Japan through the project. But kokanee and coho salmon are not nonindigenous fish and inhabit limited water areas in Hokkaido Prefecture originally.

In exchange, colored carp *Cyprinus carpio*, goldfish, sea bass *Lateolabrax japonicus*, Japanese eel, giant river prawn *Macrobrachium rosenbergii*, sewing thread *Gracilaria verrucosa*, giant ezo-scallop *Patinopecten yessoensis*, rainbow trout, and masu salmon *Oncorhynchus masou* were sent to the USSR.

Among the fish imported from the USSR, whitefish are the fastest to be produced on a commercial basis as mentioned above. Atlantic salmon reproduction was successful but because a large quantity of fish died from the high temperature of sea water in summer and from furunculosis, it was concluded that it was not suitable for aquaculture in Japan. Kokanee was introduced in 1988. Its characteristics for aquaculture have been examined for growth and maturation compared with hime salmon. Coho salmon was introduced in 1983 but died off right away, because of its infection with BKD (bacterial kidney disease).

Bester

Each 10 of bester F_1 and F_2 were introduced from the USSR to Tokai University in 1972 for the first time independently of the project. Two hundred forty-eight fry and about 300,000 fertilized eggs of bester were introduced by the project during the period 1978-1983. Initially they were received by NRIA and the Fisheries Research Institute of Mie Prefecture.

Since there was little information concerning sturgeon culture at the time of introduction, hatching and breeding temperature, food, and oxygen consumption have been studied.[4,5] Bester grew smoothly, but the most difficult problem -- reproduction -- remained.

More than 10 trials of artificial spawning had been made till 1988, but all of them were unsuccessful. The results of

Table 1. Results of the exchange project of fish fry between Japan and the USSR, 1968–88

Species introduced into Japan	Species sent to the USSR
Atlantic salmon *Salmo salar*	Colored carp *Cyprinus carpio*
Bester *Huso huso*×*Acipenser ruthenus*	Giant ezo-scallop *Painopecten yessoensis*
Coho salmon *Oncorhynchus kisutch*	Giant river prawn *Macrobrachium rosenbergii*
Kokanee salmon *Oncorhynchus nerka*	Goldfish *Carassius auratus*
Whitefish *Coregonus autummalis*,	Japanaese eel *Anguilla japonica*
C. lavaretus,	Japanese seaperch *Lateobrax japonicus*
C. muksun,	Kuruma prawn *Penaeus Japonicus*
C. peled	Masu salmon *Oncorhynchus masou*
	Rainbow trout *Oncorhynchus mykiss*
	Sewing thread *Gracilaria verrucosa*

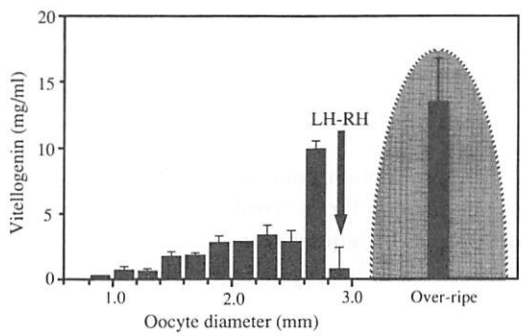

Figure 2. Relation between oocyte growth and serum vitellogenin level in bester.
Arrow indicates the best timing to induce spawning by administration of LH-RH.
Dotted line indicates the change of reabsorbed egg yolk protein level in over-ripe female serum (estimated value).

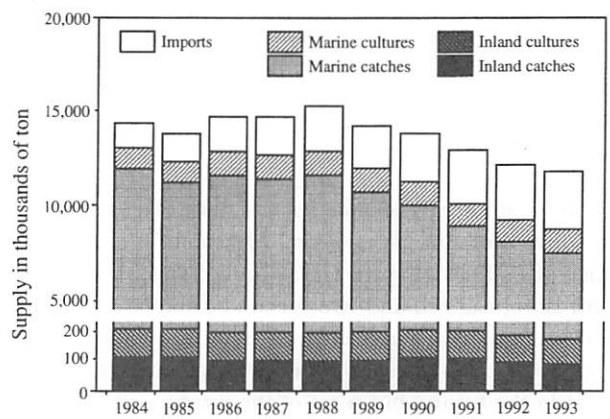

Figure 3. Component of total fish supply in Japan, 1984-93.

histological observations suggested that the gonads of the most precocious males and females attained the spermatogenesis stage at the age of two and the vitellogenesis stage at the age of five, respectively.[6] Differences in the maturational stage between individuals were also observed especially in females. The authors' group estimated that past failures in its reproduction were due to insufficient selection of the females, so we tried to develop a method to judge the maturational stage of the females more correctly.

As a result of investigations, the relation between oocyte development and serum vitellogenin (Vg) level was found. The Vg level increased concurrently with oocyte growth and decreased sharply at the end of vitellogenesis, and reabsorbed egg yolk protein appeared in serum of over-ripe females. These results showed that the best timing of hormonal treatment to induce maturation and spawning was at the Vg level (lower than 1 mg/ml) after high concentration of Vg (about 10 mg/ml) and before appearance of reabsorbed egg yolk protein (Figure 2). The first success in reproduction of bester was attained by using Vg as a maturational indicator in 1988.[7]

At present, bester have been distributed mainly from NRIA to two universities, 10 public or private research institutes, 18 aquariums, and 2 municipalities. And two companies in Miyagi Prefecture and Ibaragi Prefecture and one prefectural research institute in Miyazaki Prefecture supply sturgeon fry to more than 20 fish farms.

The remaining problem to popularize and develop sturgeon culture is to advertise sturgeon as a fish meat apropos for Japanese tastes. Because many Japanese know sturgeon as the mother of caviar, few recognize it as a fish source.

DISCUSSION

While total fish production in Japan has been decreasing since 1988, imported fish and fisheries products gain in quantity year by year (Figure 3). This increase of imports is caused not only by a decrease in domestic production and a gap in the price of the products between Japan and foreign countries, but also by the fact that domestic suppliers are not able to meet consumer demand.

Aquaculture of food fish in Japan commenced in the early 17th century with carp culture. Since then, especially after the Second World War, aquaculture in Japan has spread in number of species and area. Its development is contributed mainly to advances in the technology of aquaculture and industries surrounding aquaculture.

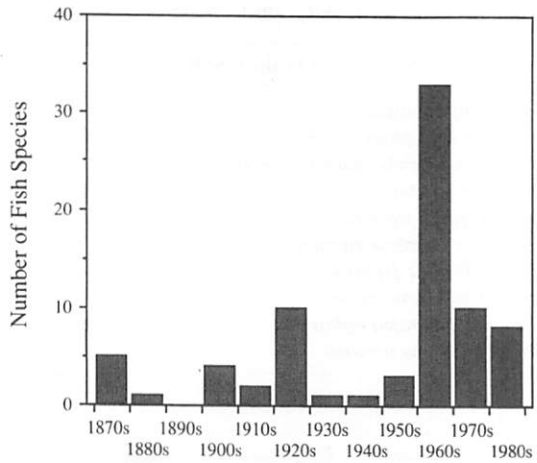

Figure 4. Number of nonindigenous fish introduced into Japan, 1877-1988.

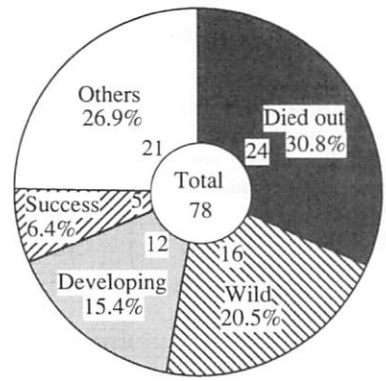

Figure 5. Result of introduction of nonindigenous fish.

Seventy-eight nonindigenous species have been introduced for aquaculture in Japan from 23 countries during the period 1877-1988 (Figure 4), but only 5 species (rainbow trout, European eel, and two species of tilapias) including coho salmon have succeeded as objects of aquaculture. Among the rest, several species are expected to be produced in the future, but most of the introduced fish failed to settle in Japan as objects of aquaculture (Figure 5).

The many failures were caused by the unfitness for Japanese taste or Japanese environments, or by technical problems to breed or reproduce them. The taste of the fish is the most important condition for success. The situation seems hopeless of improvement if the taste of the fish is not popular.

On the other hand, advances in aquaculture technology have been so remarkable in recent years that technical limitations have been solved. More varieties of fish including the fish which were not able to be cultured will be reared in the future. The restriction of the species in aquaculture has often caused overproduction of specific species. In order to correspond to a variety of consumer demand and to develop the aquaculture industry, development of new species will continue from now on.

Past examples presented here teach us that information before introduction and examinations after introduction about the taste (fitness for Japanese taste); breeding (fitness for Japanese environment); supply of fry (stable import or reproduction domestically); and diseases of the fish are important for successful introduction of nonindigenous species for aquaculture.

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Bull. Natl. Res. Inst. Aquacult., Suppl. 3 : 59–68 (1997)

THE USE OF BIOENERGETIC MEASUREMENTS TO ESTIMATE PREY CONSUMPTION, NUTRITIONAL STATUS AND THERMAL HABITAT REQUIREMENTS FOR MARINE ORGANISMS REARED IN THE SEA

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ABSTRACT

Physiological measurements can be useful in determining the suitability of organisms for mass culture in marine systems. Oxygen consumption rates of carefully acclimated animals can identify the organisms' thermal comfort zone, and estimate the metabolic costs under different thermal conditions. Conversion efficiency trials can be used to estimate a species requirements for prey by taxa and season. Temporal and geographical measurements of whole body energy content can identify the nutritional status of organisms which can provide insight into site suitability, competition from other species, seasonal feeding intensity, and interannual variation in prey availability. Examples of physiological measurements illustrating the usefulness of bioenergetics to marine aquaculture research and development programs are presented and discussed.

INTRODUCTION

Alaska is noted for its pollution free waters, extensive protected coastline and rich marine fauna. Currently aquaculture in Alaska is restricted to salmon, but there are numerous other marine fish and invertebrates that might be suited for culture in this cold water region. However, very little is known about the life history of most Alaskan species, making selection for culture problematic. Even with salmon, there are questions about the number of hatchery fry which can be introduced into the ocean without harming other species and wild fry. This paper reviews some physiological approaches that can be used to evaluate species for mass culture and examine the nutritional status of fish released into the marine environment, and their effect on competitors.

Respiration

Oxygen consumption rates can be useful in determining metabolic energy needs. This information is currently used in models that predict predation rates[1], and in the future may be used to construct models that estimate the carrying capacity of a habitat for introduced species. The flathead sole, Hippoglossoides elassodon, is a common benthic fish in the Gulf of Alaska and the southeast Bering Sea. Because of its abundance it is a key member of the food web[2] and thus is used in this report as an example of how the thermal habitat modifies metabolic energy needs.

Feeding Studies

Combining controlled feeding with growth measurements of captive fishes can be used to examine the capacity of a species for compensatory feeding, make estimates of prey consumption and conversion efficiency. A study of compensatory feeding in *Pleuronectes asper*, the yellowfin sole (formerly called *Limanda aspera*) is reviewed to explore this approach. Yellowfin sole is most abundant on the Bering Sea shelf, which is covered by ice for part of the year. The extent and duration of this ice cover shows considerable interannual variation[3], as does the boreal bottom water mass that in the fall encroaches southward on the shelf[4]. The role of temperature on the regulation of feeding and growth is poorly described for the species, but it is known that yellowfin sole feeds primarily in spring and summer[5].

The duration of ice cover and boreal bottom water affects the thermal habitat and length of its growing season. Knowing the capacity of a species for compensatory growth helps to understand interannual variations in size at age for species with seasonal impediments to feeding.

Feeding studies can be used to describe the rate of growth relative to energy consumption when a variety of prey species is fed to captive fish. Experiments with flathead sole and *Theragra chalcogramma*, the walleye pollock, are used to estimate consumption needs when different prey are available.

Somatic Energy Content

Measurements of whole body energy, combined with estimates of assimilation efficiency, can be used to quantify consumption needs. These measurements are also useful when estimating loss of a species to its predators. Examination of somatic energy content is one of the most direct and sensitive methods to quantify nutritional status and can provide insight into the suitability of a habitat to support cultured and released species. Temporal changes in the somatic energy content of yellowfin sole are reviewed to show the magnitude of seasonal changes in energy intake. In another example, geographical variations in the condition of pink salmon, *Oncorhynchys gorbuscha*, in Prince William Sound (PWS) area of Alaska are used to characterize the energy profile of fry. Hatcheries annually release about 500 million pink salmon fry into PWS. The fry must compete with themselves, wild fry, and other pelagic fish like walleye pollock, Pacific herring (*Clupea pallasi*) and capelin (*Mallotus villosis*) for prey. Theoretically rapid growth and good condition increases a fry's chances for survival because bigger fish are less susceptible to predation than smaller ones[6]. Measuring somatic energy content may evaluate growth more precisely than size and age studies.

METHODS

All the experiments reviewed in this report were previously published except for the example using pink salmon fry which is part of a project the author has just started and no published reports exist for it yet. The paper from which the reviewed material is noted in the methods section.

Respiration

The example experiment used to illustrate the use of oxygen consumption rates as an approach to estimating minimal food consumption rate for flathead sole is taken from Paul *et al.*[7]. Flathead sole used in all respiration experiments were captured by trawl in Resurrection Bay near Seward, Alaska. Experiments were done at temperatures which the species normally encounters in the Gulf of Alaska, 3 to 6℃.

Measurements of oxygen consumption relative to water temperature were accomplished with 40 to 70 g unfed flathead sole. Test temperatures ranged from 2.0 to 9.5℃. In all cases the variation of test temperature around the mean value was 0.3℃ or less. For each of four temperatures, five or six fish were acclimated to the temperature for one month. During that period they were fed Pacific herring tissue daily. Next they were acclimated to individual 23.5 l black plastic chambers for one week without food, prior to triplicate oxygen consumption measurements. Temperatures were provided by the natural seasonal thermal cycle of Resurrection Bay. All oxygen measurements were made with an electronic probe calibrated against Winkler titrations[8]. During measurements of oxygen consumption the chambers were sealed for 24 hr. Oxygen concentration was measured at the beginning and end of the 24 hrs. Oxygen levels in the experiments did not fall below 3.5 ml/l so that background oxygen levels should not have affected oxygen consumption[7]. Measurements of oxygen consumption for each fish were made on 3 separate d and the results were averaged.

Feeding Studies

Compensatory Feeding

A previous report yellowfin sole is used to illustrate the approach of estimating growth potential under restricted prey conditions[9]. Sub-adult yellowfin sole (total lengths 12-20 cm) were collected in Resurrection Bay with an otter trawl fished at 30 to 60 m depth. Each fish was weighed (g), total length measured (mm) and calculations of wet weight condition factor ($W \times 10^5$/total L^3) made.

The relationship between wet weight condition factor and specific body energy content, expressed as J/g, was determined (see Paul *et al.*[9]). The resultant equation was used to assign each of the live animals in the growth study an initial specific body energy estimate in J/g wet weight. The live sub-adult yellowfin sole were divided into four groups of four (control group) or six fish (test groups) each, and after measuring weights and total lengths, placed in 400 l tanks. All test groups contained fish with enough variety in size, or markings, so that individuals could be identified. The experiment lasted 12 weeks and measurements of size and energy content were done only at the beginning and end of the observations and not replicated. The 12 week period is approximately the length of the feeding season in ice covered habitat. Group one received food every other d for 12 weeks. Group two was starved for two weeks then fed ad libitum every other d for ten weeks. Group three was starved for four weeks and then fed every other d for eight weeks. Group four was starved for six weeks and then fed every other d for six weeks. The food

was C. pallasi fillet. Whenever fish were fed, enough food was added to the tank so that there was food remaining after 24 h. The next d after feeding, the remaining food was removed. The tanks were in a controlled temperature room at 4℃ (0.4) with light levels constant at 0.5 lu × 24 h/d. At the end of 12 weeks all fish were reweighed, measured, and calculations for wet weight gain, change in length, and specific body energy content (J/g) completed.

Growth vs. Consumption

The experiment reviewed to illustrate the approach of estimating growth relative to energy consumption rates uses flathead sole and is taken from Paul *et al.*[10]. Flathead sole were held in pairs, having weights within 10% of each other, in 400 l tanks. They were held as pairs because fish did not feed nearly as well when held alone. Fish were anesthetized with MS 222 and weighed to the nearest gram. The11 pairs of fish had mean weights ranging from 1 to 350 g. Seven of the pairs contained fish between 50-149 g which served as a standard weight range. The other four pairs were included to examine the effect of fish weight on daily weight gain. Groups of fish were fed pre-weighed herring fillets, for which the energy content was known. The energy content for samples of the food was measured with an adiabatic calorimeter. Fish were offered food daily but some pairs of fish were avid feeders while others were not. This natural variation in group feeding levels provided a variety of energy consumption levels. Experiments were done at 4℃ (0.8), a temperature the species normally encounters during the summer feeding season. Photoperiod for all experiments was 9 h of light and 15 h of darkness. Fish were acclimated at the experimental temperature for 4 weeks before the experiments began. Fish growth was calculated using the formula: $G = (\log_e W_T - \log_e W_O) \times 100/t$; where G=growth in per cent body weight per d, W_O=initial mean fish weight, W_T =final mean fish weight, and t=the duration of each experiment (always 30 d). In one pair of fish where no food was offered, G was negative and equal to the starvation weight loss. Consumption, as a percent of body weight, was calculated by summing the weight of all food actually eaten during the experiment, dividing first by 30 d, then by the initial fish weight, then by the number of fish in the group. Energy consumption was calculated by multiplying the summed weight of food by its energy value, dividing by 30 d and initial fish weight, yielding food intake as calories or J/g of fish weight/d.

Consumption of Different Prey Types

The energy content of prey varies with taxa and season; the consequences of this situation when estimating consumption rates are explored in 2 examples. Using the results of the growth vs. consumption example for flathead sole outlined above an estimate was made of how much energy they needed to consume to achieve growth rates observed in nature. Growth rates and weights of the various age classes of flathead sole from the Bering Sea were calculated in Paul *et al.*[10] using the instantaneous growth coefficient equation of Chapman[11]: $G_x = \log_e W_i - \log_e W_{i-1}/365$, where G_x is instantaneous growth coefficient, W_i is weight in the ith year, W_{i-1} is weight in the previous year to indirectly estimate prey needs. We expressed these coefficients as growth in %bw/d. Growth rate of flathead sole recalculated from fishery data conforms to the power function: growth rate (%bw/d) = 0.631 (age in years)$^{-1.16}$; $r^2 = 0.97$[10]. Once the energy-growth relationship was developed in the laboratory, the energy required to achieve that growth rate was predicted by Chapman's equation. These energy values were converted to equivalent rations of two typical prey of flathead sole: brittle stars and the walleye pollock[2]. Brittle stars are low energy food, having a energy content of ~ 2121 J/g wet weight[12]. Walleye pollock are higher energy food, having an energy content of ~ 6042 J/g wet weight[13]. Growth rates, minimal energy requirements and minimal ration requirements for these two foods were all illustrated as functions of fish age.

The other example reviewed that illustrates the effect of prey type and energy content on growth uses walleye pollock[14]. Pollock of 30-60 g were captured in Resurrection Bay and held in 800l aquaria at 5.5℃ (± 0.5). Fish were held for a minimum of one month prior to experiments to insure that they were used to captive conditions and were feeding regularly. The pollock were held in groups of two to five, with individuals in a group weighing within 10 g of each other. This approach was used because pollock are schooling fish, and when held alone they do not feed well. Growth measurements were restricted to initial weights and final weights. During measurements fish were anesthetized with MS-222. After weighing to the nearest g, fish fasted for 24 hours, were fed for 30 d, then not fed for two d. Weights were measured again on d 32.

The food types fed to pollock were C. pallasi fillets; Pacific cod fillets (*Gadus macrocephalus*); pink salmon fillet; whole amphipods (*Anisogammarus pugettensis*); whole glass shrimp (*Pasiphaea pacifica*); and whole euphausiids (*Thysanoessa raschii*). There was a single group of fish for each of the prey types. Pollock were fed once per d to satiation with pre-weighed amounts of food, and the uneaten portion was removed from the tanks after ten minutes and weighed to determine the weight ingested. While fish were offered food each d not every fish fed. Thus, the results are indicative of average growth for the group. Two groups of fish were starved for 30 d to provide data on growth at a zero caloric consumption rate. Bomb calorimetry was performed in triplicate on dried subsamples of each food type to calculate the caloric value of the food ingested.

Somatic Energy Content

The first example of using whole body energy content to determine the seasonality of growth and energy expenditure reviews a study of yellowfin sole[14]. Specimens of yellowfin sole were collected in Resurrection Bay with a 2.5 m otter trawl. Samples were collected during the 16th to the 20th of every month at the same location. Eight subsamples of juvenile and adult yellowfin sole were obtained in 1988. Fish that had well developed gonads were considered mature while those<240 mm total length with poorly developed gonads were considered juvenile.

Each yellowfin sole was weighed (g), measured (mm) and dissected to obtain: total weight (TW) for the whole body after the stomach contents were removed. Each month the whole bodies were pooled into male, female and juvenile groups. Then all the bodies in each respective category were ground together and prepared so that sex and maturity state differences in whole body energy content could be determined for each sample period. Energy values are reported as monthly estimates of J/g wet weight.

All the monthly tissue samples of a given type of yellowfin sole (adult female, male and juvenile) were pooled together and their individual weights added to obtain the wet weight (WW) of all the tissue in that group. Then they were ground and freeze-dried. Freeze-dried tissue samples were then dried again to a constant weight (0.001 g) in an oven (60℃, 48 hr.) to get tissue dry weight and reground. Wet and dry tissue weight values were used to calculate wet weight/dry weight conversions necessary for energy determination. Triplicate subsamples of all dried tissue types were analyzed in an adiabatic calorimeter for energy content.

In another example of using whole body energy content to examine the nutritional status of fish some unpublished information for pink salmon fry in Prince William Sound, Alaska is included. Somatic energy content is reported for 61 pink salmon fry captured near the Esther Island salmon hatchery on 31 May 1995. Fry from Port Gravina (n=42) and Perry Island (n=28) were captured on 27 May and 2 June 1995, respectively, for energetic analysis. The fry from Port Gravina were probably the offspring of wild spawners since there are no hatcheries in the region. The origin of the Perry Island fry could either have been hatchery or wild.

After capture the pink salmon bodies were immediately frozen, and kept frozen until analysis. In the laboratory the fish were partially thawed, just enough for handling, but not enough to lose fluids. The data gathered for every individual was standard length (SL), wet weight, dry weight, and whole body energy content that was determined by bomb calorimetry, with one burn utilizing 100% of that individual's tissues.

RESULTS AND DISCUSSION

Respiration

Measurements of oxygen consumption rates relative to water temperature for 40 to 70 g unfed flathead sole, exhibited a linear increase between 2.0 and 9.5℃ described by the equation based on mean values: $\mu l\ O_2/g/h = 3.077\ (T℃) + 1.655$; $r^2 = 0.99$ (Figure 1). The estimate of respiratory Q_{10} for sole held at 2.0 to 9.5℃ was 6.3. Based on the Figure 1 equation, for every ℃ increase (2 to 10℃) the respiratory energy needs increased by 10 to 39% (Table 1). Respiratory rate changes due to warming increased the most at the colder end of the test temperatures (Table 1).

The high Q_{10} values for flathead sole suggest that it evolved to live in cold water habitats with a narrow thermal range. In the southeastern Bering Sea flathead sole apparently select 2-4 ℃ habitat in preference to

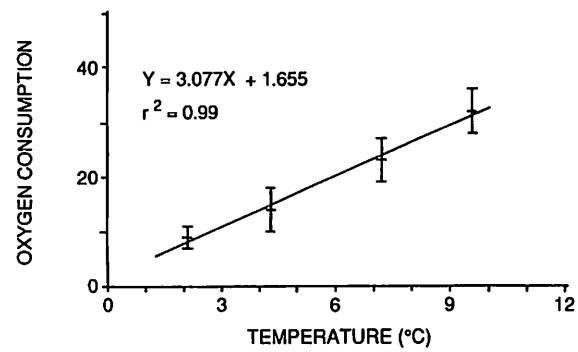

Figure 1. Specific oxygen consumption ($\mu l\ O_2/g/h$) of unfed 40-70 g *Hippoglossoides elassodon* relative to water temperature: values are mean + 1 sd.

Table 1. Changes in oxygen consumption rates of 40 to 70 g fasting flathead sole, *Hippoglossoides elassodon*.

Temperature (℃)	Oxygen Consumption $\mu l\ O_2/g/h$	Percent Change
2	7.8	–
3	10.88	39
4	13.96	28
5	17.04	22
6	20.12	18
7	23.19	15
8	26.27	13
9	29.34	11
10	32.42	10

warmer or colder strata by altering their depth distribution[15]. These observations of its respiratory biology and distribution suggest that successful culture of flathead sole would require a cold water environment without large seasonal fluctuations in temperature.

Measurement of oxygen consumption is an easy methodology to quantify the magnitude of alterations in metabolic rate due to temperature changes. It can also be used to make estimates of consumption, although these estimates are often not precise since activity levels are seldom known. Often laboratory derived measurements of conversion efficiency and growth done with feeding experiments are accomplished at only 1 or 2 temperatures, or for a restricted size range of fish. Measuring oxygen consumption can cheaply supplement feeding trial data providing scaling factors for estimating the effects of thermal change on energy needs. Measurements of oxygen consumption rate done for the whole range of temperatures a species would encounter under culture conditions can be used to identify temperatures at which respiratory stress is induced. This information would be useful in determining the temperatures to culture a species and identifying habitats into which they could be released.

Feeding Studies

Compensatory Feeding The initial mean energy content values for the test groups of yellowfin sole were estimated to be 4.4 to 4.7 kJ/g (data from Paul *et al.*[9]). The fish in all groups markedly increased their specific body energy content during the experiment. The final measured mean energy content values were 5.6, 6.0, 6.0 and 5.6 kJ/g for fish that were starved for zero, two, four, and six weeks respectively (Figure 2). These changes represented increases of energy content of 0.21 to 0.31 kJ/g wet weight over initial values. None of these increases differed significantly over the whole 12 weeks. For example, comparing the control group with the six week starvation group yielded a t value of 0.76 ($0.50 > p > 0.20$).

All the yellowfin sole in every treatment group gained weight during the 12 weeks. The yellowfin sole fed continuously and those starved for two weeks gained the most weight, an average of 25% and 24%, respectively, over initial weight. The mean increases in wet weight for fish starved for four and six weeks were similar, 16 and 15%, respectively (Figure 3). Both of these values were significantly below that exhibited by the group fed continuously ($t = 2.38$, $p < 0.05$; $t = 2.49$, $p < 0.05$, respectively). Because of this disparity in weight gain, the total body energy content of a 50 g fish would have increased by 58% in the group starved for two weeks vs. 46% and 35% for those starved for four and six weeks. None of the treatment groups exhibited significant differences in terms of growth in length over the whole twelve weeks (Figure 3). The most divergent values, the control and two week starvation groups, yielded a t value of 1.61 ($0.20 > p > 0.10$).

Studies of compensatory feeding are often undertaken to asses the potential for minimizing the cost of feeding a cultured species. Alternating bouts of three weeks starvation and three weeks feeding in rainbow trout (*Oncorhynchus mykiss*) produced weight gains equivalent to those of continuously fed controls[16]. Such compensatory

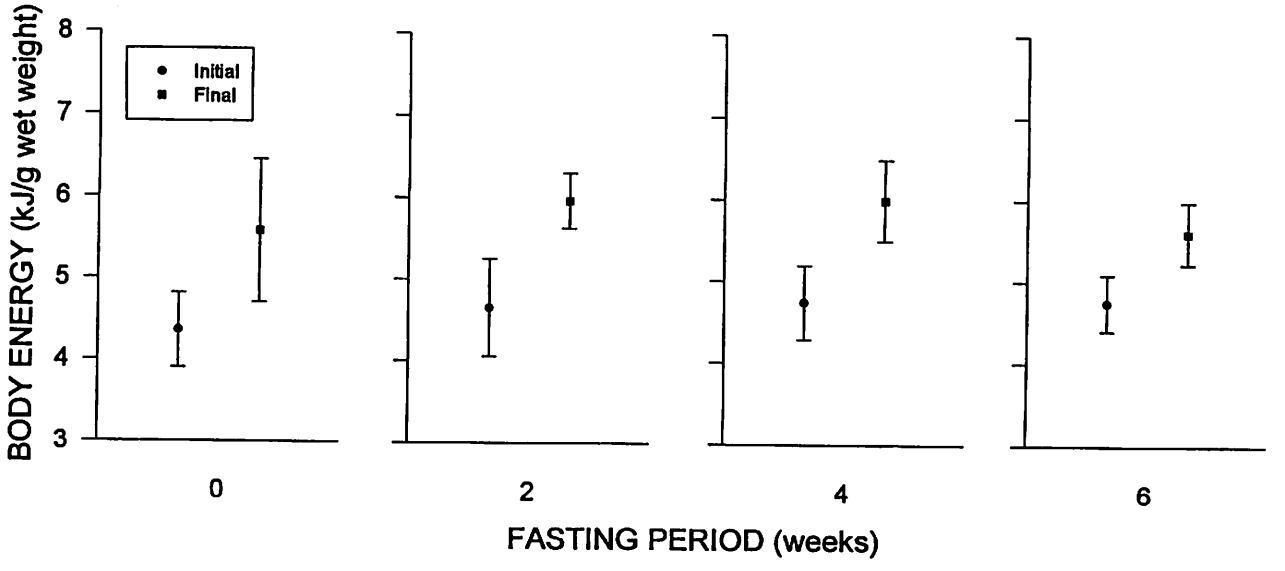

Figure 2. Change in whole body energy content (kJ/g) in sub-adult *Pleuronectes asper* starved for varying periods at the beginning of a 12 week study then fed to excess.

growth regimes result in no significant differences in body water, fat, protein or ash in comparison to continuously fed control fish[17]. The experiments with yellowfin sole indicated that lengthy periods of starvation caused weight loss that could not be recouped by subsequent periods of feeding to satiation. Within the constraints of the experiment, yellowfin sole were unable to compensate, in terms of weight, for periods of starvation beyond two weeks. Feeding regimes of captive trout are often manipulated to use that animals' ability to undertake compensatory feeding to maximize growth and minimize consumption. This procedure would not work as well with yellowfin sole because of its limited capacity for compensatory feeding.

The study of compensatory feeding provides insight in the growth patterns of a species. All the groups of yellowfin sole had similar water content and increases in both specific energy content and length over the 12 weeks of study suggesting that when food was limited sub-adults grew in length preferentially to weight. This preferential allocation of energy to growing lengthwise may explain some of the naturally occurring interannual variation in length-weight-age relationships reported in this species. If cultured yellowfin sole released into natural habitats were found to be preferentially growing in length it might indicate food limitation.

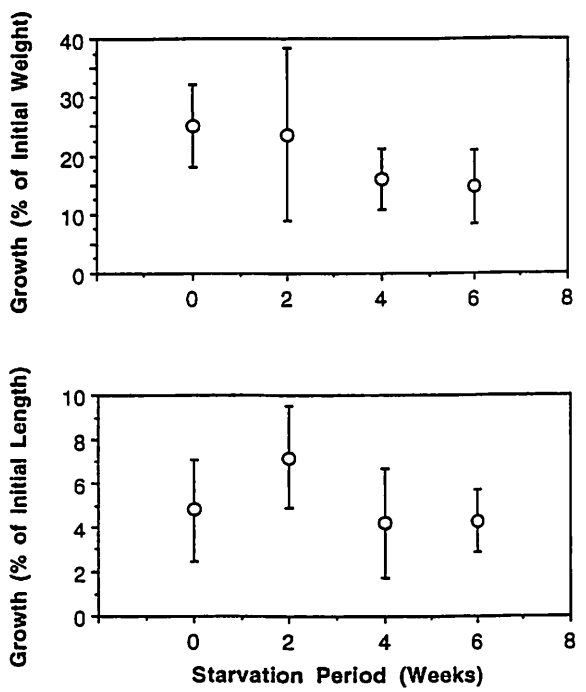

Figure 3. Change in wet weight (upper) and length (lower) in sub-adult *Pleuronectes asper* starved for varying periods at the beginning of a 12 week study then fed to excess.

Growth vs. Consumption

Growth of captive flathead sole was linearly related to energy consumption by the equation: Growth (%bw/d) $=0.006$ (Consumption J/g/d) -0.130; $r^2=0.8$ (Figure 4). The r^2 value (0.8), and the fit of the data points, suggests that growth for 1-359 g fish was independent of size when weight gain was expressed as a function of consumption in J/g/d (Figure 4). At 4.0℃ the estimated maintenance ration based on feeding and growth observations was 21.7 J /g/d.

The energy requirement to achieve the growth rate equation noted in the methods [growth (%bw/d) $=0.631$ (age in years)$^{-1.16}$] conformed to a power function: Energy requirement (J/g/d) $=96.8$ (age in years)$^{-0.52}$; r^2 $=0.93$. Converting these energy requirements to minimal rations of brittle stars and walleye pollock necessary to achieve the observed growth rates yielded the power curves seen in Figure 5. Estimates for daily ration necessary to achieve growth rates observed in the Bering Sea were calculated to be ~ 0.4 to 6.2% bw/d depending on fish size or age and prey energy content (Figure 5). Using low and high energy prey, we estimate the minimal requirement of flathead sole to range from 2.2 to 6.2%bw/d in its first year. The minimal rations required to achieve observed growth declines with increased age and body size so that by age 16, they range from 0.4 to 1.2%bw/d.

The preliminary type of consumption model in Figure 5 predicts the minimal ration required by any age (or weight) fish. This type of bioenergetic exercise would be useful in determining the carrying capacity of a habitat for cultured species and natural inhabitants. A habitat carrying capacity model requires a good knowledge of prey communities, their seasonal availability and energy content. In most Alaskan regions this basic information is not

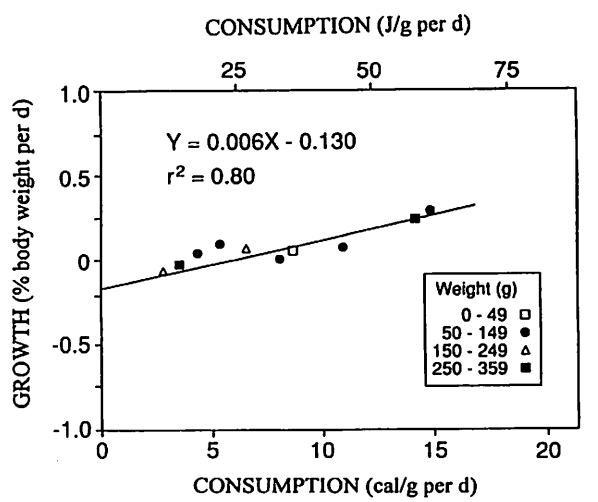

Figure 4. Growth (% body weight/d) for *Hippoglossoides elassodon* of 1 to 359 g at 4.0℃ relative to energy consumption. Equation relates growth and consumption in J g-1.

available.

Knowing the assimilation efficiency of a species is important in determining its suitability for profitable culture. Flathead sole between 1 and 359 g were similarly efficient at converting energy to body weight, as indicated by the linearity and the fit of the data. Most of the prior bioenergetic studies of flatfish were done at warmer temperatures. Growth data exists for plaice (*Pleuronectes platessa*) and common dab (*Limanda limanda*) at 10℃[18] and those results suggest that the maintenance rations for the two species would be 79 and 104 J/g/d for plaice and dab, respectively. These maintenance rations were about 3.5 and 4.7 times that of flathead sole at 4℃. Much of this difference is probably attributable to the thermal habitat, but this is speculation without further studies. However, it does suggest that there may be large differences in the maintenance ration of different species of flatfish, a topic that needs study to aid in the selection of species to culture.

Consumption of Different Prey Types

Figure 6 shows the linear relationship between consumption of different prey and growth in walleye pollock: Growth (%bw/d) = 0.044 Consumption (cal/g/d) $^{-0.343}$; r^2=0.95. The poorest growth was shown by fish fed amphipods, the prey with the lowest energy content. The best growth was obtained by groups fed herring fillet which had the highest energy content per unit weight. The results demonstrate that pollock convert the energy of crustacean and fish tissues with similar efficiency.

Expected changes in growth rate as different prey species are utilized could also be predicted in growth expectancy models. In the pelagic community the dominant taxa of plankton and forage fish often changes with season. What type of prey is being eaten has some effect on consumption rate. For example in laboratory studies of walleye pollock maintenance rations for adults held at 7.2 ℃ were 0.8% bw/d[19] when fed walleye pollock fillet (18 kJ/g[13]) vs. 0.26% bw/d at 5℃ when fed herring fillet (26 kJ/g,[13]). The thermal regime probably accounts for some of the difference in maintenance ration based on weight of food consumed. Variations in energy content of the food can explain much of the large difference in these estimates of weight-based maintenance ration. The maintenance ration for adult pollock based on energy intake is about 20 J/g/d at 5℃[13]. With a moisture content of 80%, pollock fillet has an energy content of around 3.6 kJ/g[13]. A 650 g pollock consuming 20 J/g/d would then eat 3.6 g or 0.6% bw/d, a much higher consumption rate than the 0.26% bw/d for fish fed on herring[13]. If those fish ate euphausiids (2.5 kJ/g wet wt.) consumption would be 5.2 g or 0.8% bw/d. Thus, rations are best expressed in energy units rather than as a percentage of body weight and habitat carrying capacity estimates must account for prey energy content.

Somatic Energy Content

Yellowfin sole

Figure 7 provides the seasonal cycle of somatic energy content for yellowfin sole in Resurrection Bay, Alaska. Whole body energy content of adult female yellowfin sole was near 4376 J/g in January and declined through May to about 3472 J/g. Between May and June the total energy content increased by 28% and remained near high values into September. The whole body energy content of adult males was similar to females, lowest in May and increasing by 35% between May and June. Whole body energy values were high from June through September. Whole

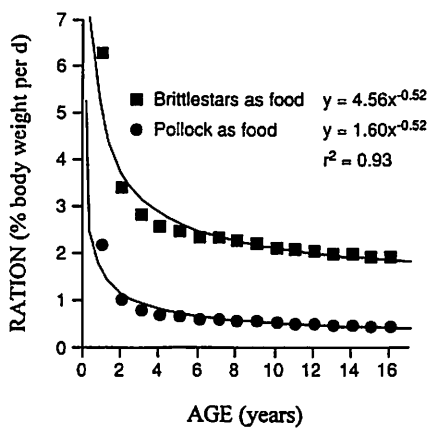

Figure 5. Minimal rations of brittle stars and walleye pollock for *Hippoglossoides elassodon* to achieve observed growth rates in the Bering Sea.

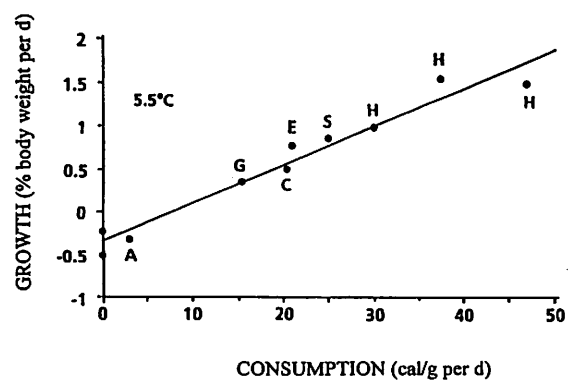

Figure 6. Growth (% body weight/d) of juvenile pollock held at 5.5℃ as a function of energy consumption (calories/g of fish weight/d). Food types of the different groups of fish were amphipods (A), glass shrimp (G), euphausiids (E), cod fillet (C) , salmon fillet (S), and herring fillet (H).

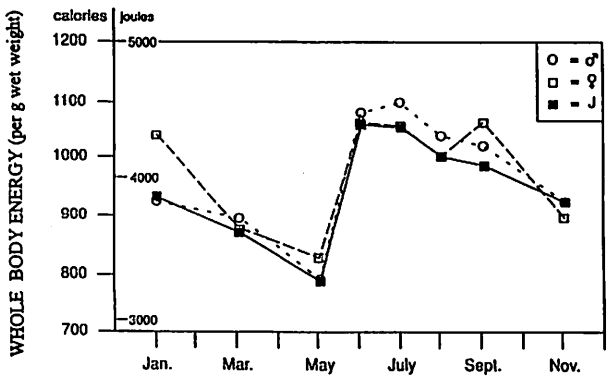

Figure 7. Whole body energy content (wet wt) for yellowfin sole *Pleuronectes asper* collected in the Gulf of Alaska, adult females (□), males (○) and juveniles (■).

body energy content was about 10% lower during winter. The seasonal whole body energy content changes measured in juveniles followed the same trends as the mature fish, and had similar values as adult fish. Energy values for their whole bodies were 3326 J/g wet weight in May then rapidly increased by 33% to 4439 J/g wet weight in June. It remained near this level through August and then began to decline in winter. These seasonal variations in somatic energy content must be reflections of temporal feeding patterns; during winter energy intake obviously must be less than metabolic needs. The 28% increase in body energy for yellowfin sole between mid May and mid June indicates that much of the annual energy acquisition takes place in a very short time period of intense feeding.

The study of seasonal changes in somatic energy content would be useful in determining when to introduce cultured species into natural habitats and times to harvest them. The seasonal samples of somatic energy content of yellowfin sole of all stages of maturity exhibited an annual cycle with active energy accumulation and growth from May through September. Thereafter until the following May, stored energy seemed to be used for metabolic and reproductive needs. The spring and summer energy storage strategy used by yellowfin sole is similar to other northern flatfish species[5,20-21].

If flatfish like yellowfin sole were cultured and then released into a natural habitat for rearing, loss to predators must be considered to determine project feasibility. The seasonal variation in whole body energy varies up to 35%. Thus, when modelling loss of yellowfin sole to predators, neglecting the seasonal aspect of body energy will cause considerable errors in consumption estimates since predators may adjust consumption rates to prey energy content.

Pink Salmon Fry

The somatic energy content of pink salmon fry exhibited marked regional differences in the levels of energy stores (Figure 8). At the Esther Island hatchery site the fry's' somatic energy content averaged 3.2 kJ/g wet wt and the mean SL was 34 mm. This was the area where fry were the most abundant because of the large hatchery. Perry Island fry had an average somatic energy content of 3.6 kJ /g wet wt, and a mean SL of 31 mm. According to unpublished Alaska Department of Fish and Game reports fry were less numerous there than at the hatchery site. At Port Gravina, where there were only a few fry produced by natural spawners, comparative values were 4.4 kJ, and 42 mm SL. Among the Port Gravina fry the highest level of whole body energy observed was 5.2 kJ/g wet wt. A Mann-Whitney Rank Sum Test indicted that the somatic energy content of fry from Esther Island and Perry Island were significantly different ($P=0.0001$). A similar comparison of Perry Island and Port Gravina energy values also indicated that they were statistically different. It appears that the number of fry in an area may modify the amount of energy they consume.

The example shown for pink salmon fry suggest that measurements of somatic energy content can be used to determine how many fry can be added to a habitat before food availability limits growth. Pink salmon fry tend to

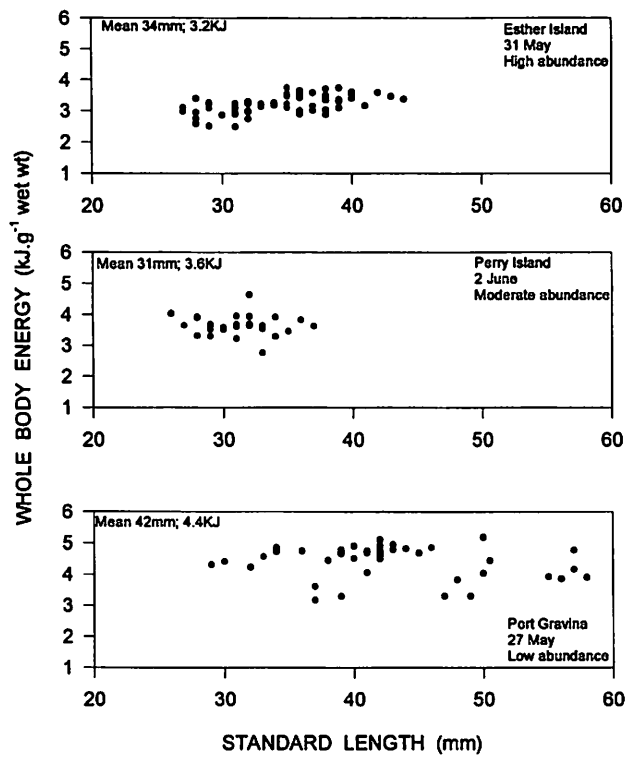

Figure 8. Whole body energy content (wet wt) for pink salmon fry *Oncorhynchys gorbuscha* collected from three sites in Prince William Sound, Alaska.

store surplus energy as lipids when food is not limiting[22]. The highest somatic energy content measures in this study were 4 to 5 kJ/g wet wt. This suggests that fish with whole body tissues >4 kJ/g were consuming enough food to grow and store energy. Few of the Esther Island and none of the Perry Island fish had built their energy stores to ≥4 kJ/g, so it appears that at the two study sites where hatchery fry competed with wild fry, feeding levels were lower than optimal. The best fed fry in our limited sampling had energy measures of 4 to 5 kJ/g wet wt implying that one should commonly see values this high if food is not limiting energy storage. Currently we have no idea what the regional or interannual differences in whole body energy content might be, so future measurements need to be done to determine what the energetic profile of well fed fry looks like. To fully understand the growth process concurrent measurements of prey biomass also needs to be collected. The measurement of somatic energy content appears to be a diagnostic measure of nutritional status that can be used to examine the fate of fish released into the sea.

General Conclusions

The examples of bioenergetics reviewed in this report show how oxygen consumption rates can be used to identify the organisms' thermal tolerance and minimum metabolic energy needs. Conversion efficiency trials can be used to estimate a species requirements for prey by taxa and season. Temporal and geographical measurements of whole body energy content can identify the nutritional status and seasonal feeding patterns of organisms in natural and culture conditions. These basic physiological measurements are cost effective ways to screen Alaska's many marine species for their suitability for culture.

If aquaculture is to be stainable and co-exist with bio-diversity the reliance on single species biological studies needs to be abandoned in favor of ecosystem studies. Aquaculture specialists must join with oceanographers, ecologists, fisheries biologists, environmental or pollution biologists and eco-system modelers to develop management strategies that lead to sustainable harvests in healthy ecosystems.

ACKNOWLEDGMENTS

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GOVERNMENTAL RESEARCH FUNDING AND POLICY FOR BIOTECHNOLOGY IN AQUACULTURE

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ABSTRACT

In recent years, new techniques using biotechnology have been introduced in order to enhance the production of aquaculture. Currently undergoing research projects on biotechnology and the government policy toward its application to aquaculture are briefly presented.

INTRODUCTION

Japanese capture fisheries are now facing severe circumstances such as fewer young fishermen, lower fish price and lower level of fish stock. Under such circumstances, aquaculture is becoming a large part of the fishery industry (Figure 1). Many new technologies have been developed in Japan to enhance the productivity and to improve the quality of aquaculture. One of them is biotechnology. It is expected to be a key technology in the future.

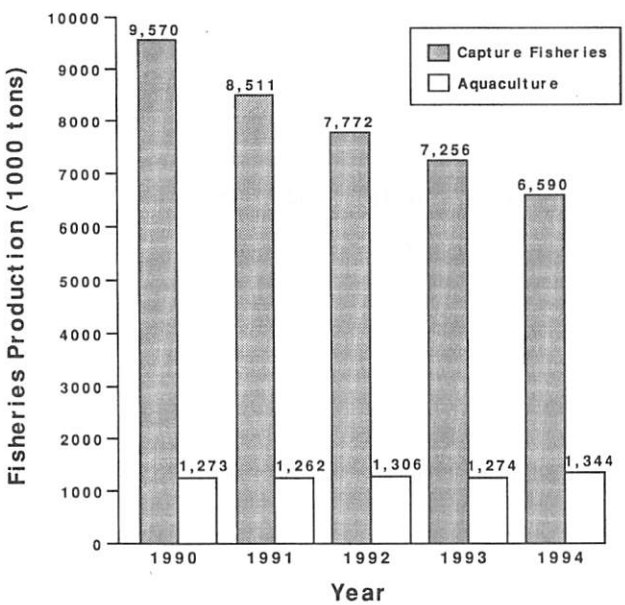

Figure 1. Annual variations in catch

CURRENT STATUS OF BIOTECHNOLOGY

Biotechnology, in this context, refers to a technique that utilizes to make products and to extract services from living organisms and their components. A broad interpretation of biotechnology includes all technologies involving organisms which can lead to a better quality of aquaculture. Some examples are traditional technologies such as selective breeding and newer techniques such as gene transfer and frozen storage of genetic material. All the established methods should continue to be an important part of the aquaculture industry. Newly developed technology may further permit an increase in the production and may bring some benefits to fishermen. However, potential adverse effects must be evaluated carefully before its application.

RESEARCH PROJECTS

In the field of biotechnology for aquaculture in Japan, the following three techniques need to be established.

(1) Recombinant DNA techniques: DNA cloning of useful genes, gene transfer, etc.

(2) Chromosome manipulation techniques: parthenogenesis, diploidization of genome, sterility, etc.

(3) Technology of gene conservation for useful strain

The Fisheries Agency of Japan promotes the following research projects. As these projects may be difficult for private sectors to do technically and financially, they are mainly carried out in national and prefectural research institutes. The total funding in 1996 is about 2.3 million dollars.

(1) Projects on recombinant DNA techniques

(a) To clear functional mechanism on growth hormone of aquatic organisms which are bases of producing useful strain

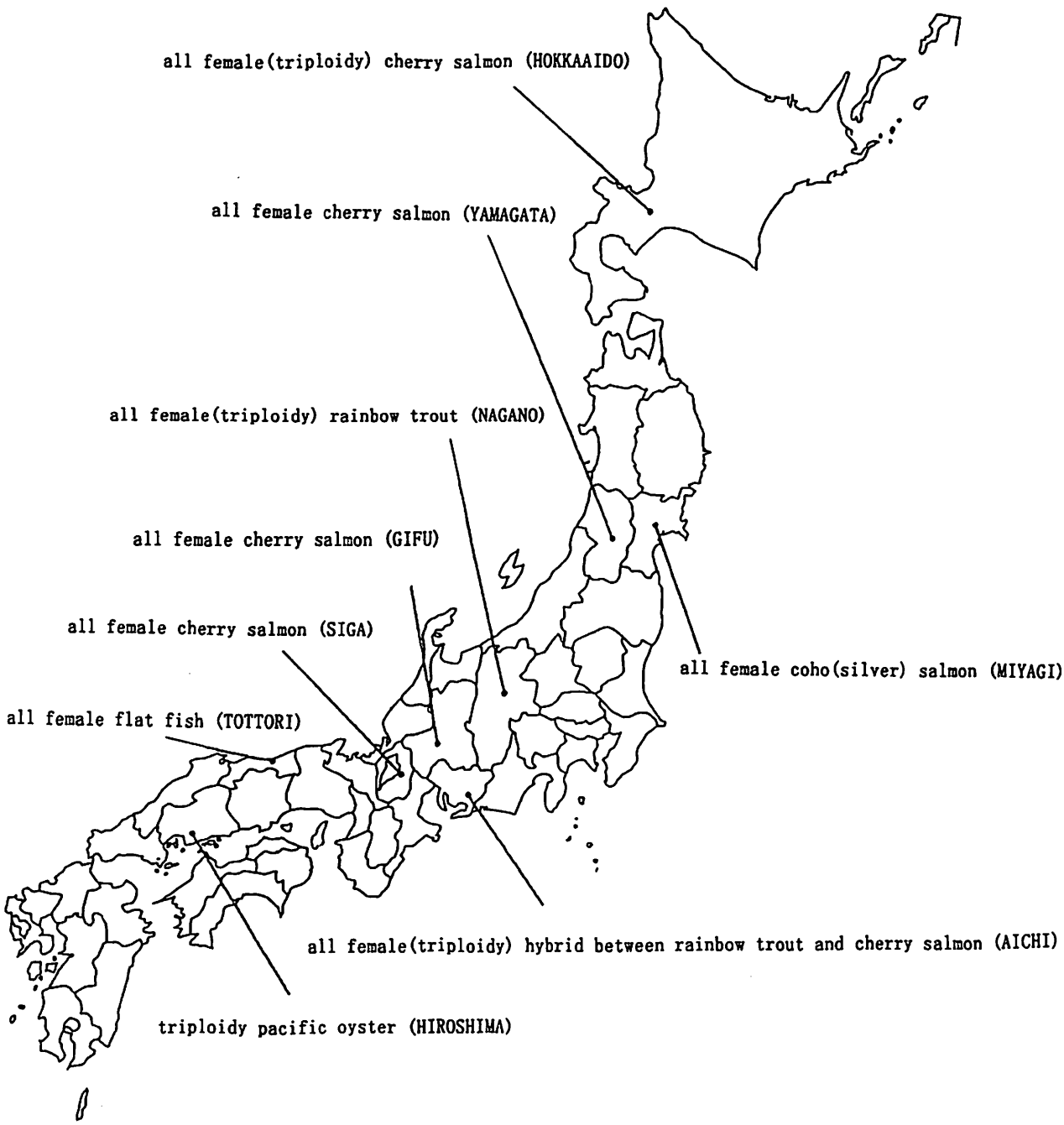

Figure 2. Fish approved by the Guidelines for the Utilization of Triploid Fish and Others

(b) To develop ecological evaluation of genetically modified organisms

(2) Projects on chromosome manipulation techniques
  (a) To develop production techniques of all female and polyploidy (triploidy) whose species are used for local industry
  (b) To develop mass production techniques of all female and polyploidy (triploidy) for aquaculture

(3) Projects on gene conservation techniques
  (a) On frozen storage of genetic materials (gametes) to conserve genetic diversity in wild ecosystem
  (b) On evaluation of methodology of genetic diversity by DNA analysis

POLICY

Japanese policy and regulations regarding biotechnology have been established in response to risk and safety issues that may arise in aquaculture. One example is the triploid technology. The Fisheries Agency of Japan set up the "Guidelines for the Utilization of Triploid Fish and Others" in 1992 for the appropriate application of this technology in aquaculture (Figure 2). In recent years, triploid and female fish populations in Japan share a large part of the aquaculture production of some fish species such as rainbow trout, flatfish, salmon and oysters. (Figure 3). Many genetically modified aquatic organisms, however, do not fall under the umbrella of any legislation. The enumerated characteristics in the Guidelines should be thoroughly examined before the practical application to such fish species.

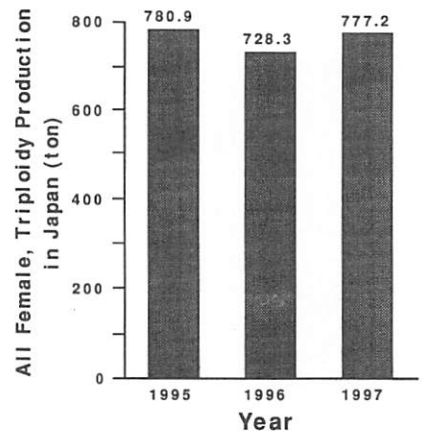

Figure 3. All female triploidy production in Japan

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COMPARISON OF THE GENETIC VARIABILITY BETWEEN WILD AND ARTIFICIALLY RAISED JAPANESE FLOUNDER (*PARALICHTHYS OLIVACEUS*)

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EXTENDED ABSTRACT

The Japanese flounder (Paralichthys olivaceus) is one of the most important fish for coastal fisheries, and more than 20 million artificially raised seeds have been released to enhance the stock size in recent years.[1] It has been reported that the artificially raised seeds are inferior to wild fish in genetic variability[2-5] and genotype composition of the wild fish could be changed rapidly by the successive release of seed.[6,7] The difference in genetic variability between wild fish and artificial seed must be understood and the development of a technique that produce seed as genetically variable as wild fish is needed. In this study, the genetic variability of wild Japanese flounder and artificially raised seed was examined by nucleotide sequences of the control region in the mitochondrial DNA. A total of 390 base pairs (bp) in the first half of the mitochondrial DNA control region was sequenced and aligned. The extremely high variability was found in the sequence of the wild flounder and each of the wild flounder analyzed had its own unique sequence. On the other hand, the number of haplotypes and nucleotide diversity of the artificial seed was much more restricted compared with the wild flounder, though the artificial seed from wild parents were superior to those from the artificially raised parents in these indexes. It was estimated that only a part of the female fish (4 - 20%) in the hatchery contributed as mothers of the seed in single egg collections. The number of contributed female fish was improved by the multiple egg collections.

In Japan, most of the hatcheries use the fish that were selected and reared generation to generation for parents, because this is easier than keeping wild fish. Furthermore, to reduce the costs and working time, a lot of effort has been made to minimize the number of egg collections by iproving the survival rates. Producing seed in this manner has reduced the genetic variability of seeds. The production of seed should be improved to conserve the genetic variability of the wild Japanese flounder.

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A NATURAL TRACKING METHOD FOR SUMMER FLOUNDER, *PARALICHTHYS DENTATUS*, IN STOCK ENHANCEMENT PROGRAMS

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ABSTRACT

The recent development of commercial summer flounder (*Paralichthys dentatus*) farming in the northeastern United States has resulted in the large scale production of summer flounder fry and fingerlings. This development, coupled with the collapse of the wild stock, raises the potential for stock enhancement. A critical concern of any release program is the ability to track the effectiveness of the program. A means of effectively tagging fish with an externally visible tag is required. Conventional tagging methods are unsuitable for small juvenile fish. One approach successfully used in Japan with olive flounder, *Paralichthys olivaceous*, has been to release fish with abnormal pigmentation markings. A percentage of summer flounder produced in commercial hatcheries develop skin discolorations--albinism on the eyed side or ambicoloration on the blind side. In this study, the potential for using these discolored fish in stocking programs on the east coast of the United States is reviewed. While both conditions would result in ready identification on board commercial harvesting vessels, it is recommended that fish with ambicoloration on the blind side would be most effective because their use would minimize the concern of increased susceptibility to predation of albino-colored fish.

DISCUSSION

The recent development of commercial summer flounder (*Paralichthys dentatus*) farming in the northeastern United States has resulted in the large scale production of summer flounder fry and fingerlings. This development, coupled with the collapse of the wild stock, raises the potential for stock enhancement. There are many critical biological, ecological, social and economic factors that must be studied and debated at a national level before any release program could be undertaken. There is, however, the unique opportunity to learn from a similar well-established program: the olive flounder (*Paralichthys olivaceous*) stocking program in Japan. Japan has been rearing juvenile olive flounder, releasing them into the wild and tracking the resulting effect on commercial landings since at least 1981. A number of papers have reported on this practice. While the purpose of this paper is to examine one specific issue of importance in an evaluation of a summer flounder stock enhancement program, an effort has been made to collect and review key literature on the Japanese olive flounder stock enhancement program. While not exhaustive, the attached bibliography lists a number of key papers presented on the subject.

A critical concern of any release program is the ability to track the effectiveness of the program. A means of effectively tagging fish with an externally visible tag is required. Artificially tagging fish is expensive, can be impractical for very small fish, and tags can be lost. One approach successfully used in Japan with olive flounder has been to release fish with abnormal pigmentation markings. A product of rearing flatfish in captivity is that a certain percentage of fish are produced with abnormal markings. Albinism is a lack of pigmentation on the eyed side and ambicoloration is a darkening of the normally white colored blind side (underside). Sproul and Tominaga[1] mention using fish with partial albinism as 'natural' tags. Kitada et al.[2] state that released fish are identified by dark markings (ambicoloration) on the blind side. These studies state that these abnormal markings have allowed for a thorough analysis of the survival and subsequent landings of hatchery-reared fish. Sproul and Tominaga[1] present a favorable economic analysis of the olive flounder stock enhancement program, presenting that the cost to the government of rearing flounder is positively offset by the increase in economic activity as a result of the landing of fish that were released into the wild. While the authors did not include the capital cost of building the government hatcheries, they only used the landed value of the fish in measuring the benefit. It is well reported that for every

dollar of landed value of seafood there is a significant additional positive economic impact (as much as three to four times) in value added to processing, distribution and resales. Thus it is likely, even with amortization of the capital investment in hatchery capacity, that the economic benefit to the local coastal community is positive.

Researchers have now discovered the cause of most discoloration in hatchery-reared flatfish and it is now possible to produce hatchlings that are correctly pigmented. The factors are largely nutritional, and larval feeding programs now produce quite high percentages of properly pigmented fish. In the United States, summer flounder currently produced for grow-out are sorted and any discolored or otherwise malformed fish are culled out of the stock. It would however, be possible to use these fish, or these fish as a known percentage of total fish released, for release programs. In commercial ongrowing, fish that display varying degrees of albinism appear to be otherwise healthy and grow at the same rate and sometimes even a faster rate than normally pigmented fish of the same class. Fish displaying ambicoloration do not appear to grow at any different rate than others.

While both types of fish could be used as natural markers and would be easily identified by commercial fishermen harvesting the fish from the wild, there are consideration in determining which condition, if either, would be preferred. First, it is possible that fish with albinism may be more susceptible to predation. If exposed, the white upper surface may draw attention. However, summer flounder spend most of their non-feeding time in the wild partially or wholly covered with sand, so fish with albinism may survive at a similar rate as normally pigmented fish. Albinism is a condition where the fish lack pigment cells, and as far as is known to date, is irreversible. Ambicoloration of the blind side, however, is not as well understood. To the best of these authors knowledge, researchers in the United States do not yet know the cause of the condition in hatchery-reared fish and also do not know if it is reversible. Two studies have been done investigating ambicoloration in summer flounder.[3,4] These studies examined the effect of substrate and tank color on ambicoloration. However, there has not been any investigation on whether fish exhibiting ambicoloration while in a captive environment will reverse the condition in the wild. If reversible, it may be that fish released with dark markings on the blind side may not be distinguishable from wild hatched fish over time. If, however, the ambicoloration is permanent, this marking may prove to be a more suitable means of marking hatchery-released fish. Clearly, ambicolored fish will not have the same concern of increased risk of predation as fish exhibiting albinism. Ambicolored fish would also be easily identified during harvesting and processing, as summer flounder are now packed blind side up for presentation.

While many other factors influence the effectiveness and value of a stock enhancement program and should be further investigated, it appears likely that a hatchery-induced 'natural' marker could be used with summer flounder in a stock enhancement program. The clear benefit is that unlike other stock enhancement programs tried unsuccessfully in the past in the United States, summer flounder stock enhancement could be accurately tracked and an evaluation of its effectiveness measured.

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ECOLOGICAL DIVERSITIES AND STOCK STRUCTURE OF THE FLOUNDER IN THE SEA OF JAPAN IN RELATION TO STOCK ENHANCEMENT

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ABSTRACT

To prevent negative impact of stock enhancement on the structure of the wild population, we have conducted research on stock structure from morphological, ecological, physiological and genetic aspects since 1991. Information on geographic differences in ecological aspects were collected from prefectural fisheries literature. Samplings for settled juveniles were conducted from the southern to northern range limit along the coast of the Sea of Japan in 1991 and 1994. Juvenile density was one-order lower in the northern Sea of Japan compared with that of the southern area. Juvenile growth between about 30 and 60mm in standard length was significantly higher in the north than in the south when compared at the same water temperature range. Meristic counts (dorsal and anal fin-ray numbers) obtained from flounder sampled in 1991 provided evidence of the existence of northern and southern stocks separated by the Noto Peninsula. More comprehensive sampling in 1994 suggests the existence of two additional stocks associated with the islands of Kyushu and Hokkaido. Sequencing analyses of the control region of the mitochondrial DNA showed this region to be highly variable, and suggested little but significant differentiations among some geographical populations. The hypothesized four stocks are going to be examined for discontinuity in hatch and settling dates provided from otolith microstructure analysis, long-term fluctuation of juvenile abundance, growth potential relative to water temperature, critical temperature for sexual differentiation and the effect of water temperature on fin ray formation. Geographic differences in ecology and the possibility of separate stocks suggest that careful attention should be paid to stock structure as we proceed to a more advanced stage of sea-farming.

INTRODUCTION

The Japanese flounder (*Paralichthys olivaceus*) is commonly distributed along the coast of Japan and is a valuable commercial species in the Sea of Japan. In Japan mass seedling production allows efforts to enhance the Japanese flounder population by mass-release of hatchery-raised juveniles. The total annual release exceeds 21 million juveniles, and million-order release has been realized in several prefectures. Although this type of stock enhancement, called sea-farming in Japan, still has various technical and basic problems, released flounder have matured in the wild and presumably have interbred with the wild population.[1)] The Japanese sea-farming project has been internationally recognized as a promising approach that could aid in the recovery of overexploited fisheries resources. On the other hand it has been criticized as its impact on biodiversity and the coastal ecosystem is not well understood.

Recently more attention has been placed on the quality of the seedlings including genetic variability. Several research projects which aim to develop the production of genetically diverse seedlings, and to evaluate the effects of releases on the ecosystem have been initiated. An urgent need for information on the stock structure of the flounder around Japan exists as its detailed knowledge is required as an essential biological base for the stock enhancement project.[2)] To approach this goal, comprehensive research is being conducted in ecological, morphological and molecular genetic aspects by Kyoto University and Fukui Prefectural University. The main purpose of this article is to show geographical and ecological differences and the possible existence of a complex stock structure for the Japanese flounder. In addition we want to stress the importance of conducting a comprehensive ecophysiological study as the most fundamental basis for suc-

cessful stock enhancement. Preliminary results from the entire distribution range in the Sea of Japan are briefly combined based on the original papers.[3-10]

MATERIALS AND METHODS

The metamorphosing Japanese flounder larvae migrate from offshore to nearshore and settle on the sandy bottom which is utilized as a juvenile nursery area for several months.[10,11] A large volume of information on juvenile distribution, abundance and growth has been accumulated in the coastal waters of the Sea of Japan. The ecological information collected mainly by each prefecture was used for analyzing the ecological differences. Latitudinal comparison in water temperature is based primarily on the oceanographic data base published by the National Japan Sea Fisheries Research Institute.[12] Direct samplings for juveniles were performed at each prefecture by a small beam trawl (1.5m mouth-size) along the coastal line from Fukuoka to Aomori in 1991 and from Kagoshima to Hokkaido in 1994 (Figure 1). Samples collected in 1991 were preserved in 10% formalin to analyze geographical variations in the meristic counts and 1994 samples were preserved in 99% ethanol for multiple usages: meristic counting, ageing by otolith microstructure and genetic analysis using mitochondorial DNA.

Meristic counts (dorsal and anal fin-rays and vertebrae) were done using soft X-ray photographs for juveniles larger than 20mm standard length (SL) and double staining method for smaller juveniles as described by Tanaka et al.[6] Daily increments on the sagittal otoliths were counted to estimate the settling date using the method developed by Goto et al.[7] Mitochondrial DNA analyses were done by using PCR-mediated amplification and sequencing of its control region as described by Fujii and Nishida[9] and Ohkawa et al.[10] Larval rearing experiments were conducted to determine the effect of water temperature on meristic counts in 1991 and 1994. The larvae were reared at three or four different temperature regimes from pre-flexion stage beyond metamorphosis as described by Seikai et al.[8] In order to determine the precise effects of temperature on cloned flounder larvae produced in the Tottori Prefectural Fisheries Experiment Station were used as experimental animals in 1995.

RESULTS

Latitudinal differences in the life history traits

Spawning season

The gonad somatic index (GSI) peaks in February in the southern Kyushu and in March in the northern Kyushu when the water temperature is lowest. The peak GSI shifts from early spring to early summer with an increase in latitude and occurs in summer in Hokkaido (Figure 2, Tanaka et al.[3]). A discontinuous shift can be seen between Kyoto and Niigata. This implies that the spawning season extends over the early half of the year

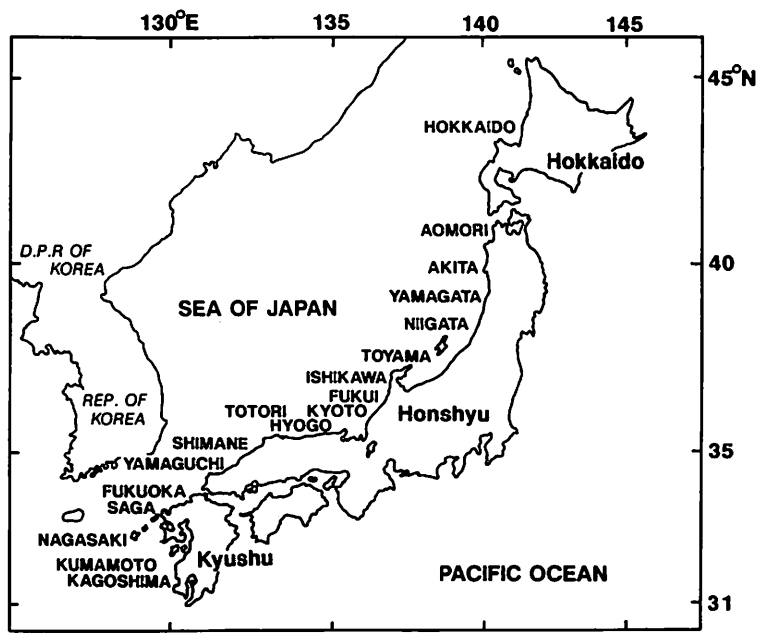

Figure 1. Map showing prefectures where samplings for the Japanese flounder juveniles were conducted: 1991 from Fukuoka to Aomori and 1994 from Kagoshima to Hokkaido.

with a successive shift from south to north.

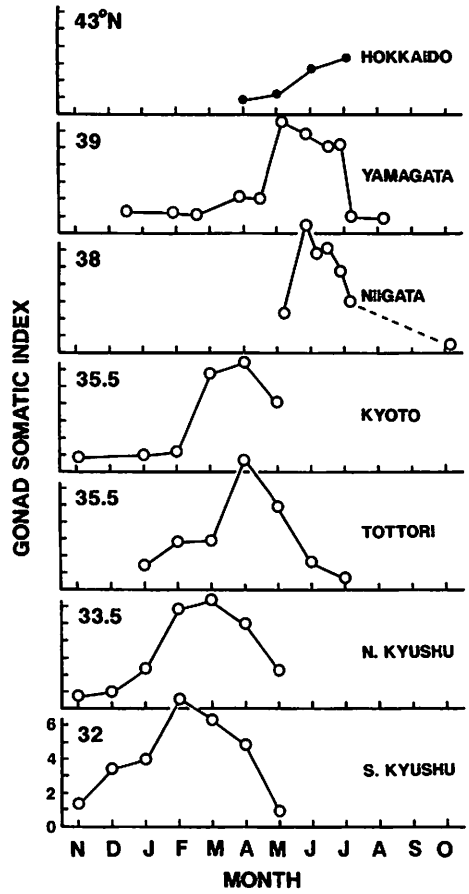

Figure 2. Seasonal changes in gonad somatic index (GSI) of the Japanese flounder at seven locations of different latitude. Data from Hokkaido are another maturation index. (Tanaka et al.[3]).

Table 1. Average density of juvenile flounder at latitudinally different nurseries along the Sea of Japan and adjacent coastal waters estimated from anonymous data sources (Tanaka et al.[3]).

Latitude	Location	Density* (per 1000m^2)	Year (No. of year)
43.2°N	HOKKAIDO	5.8	1988-1991 (4)
40.8	AOMORI	10.7	1980-1989 (10)
40.0	AKITA	1.3	1988 (1)
38.1	NIIGATA (Iw)	2.9	1981-1993 (13)
37.9	NIIGATA (Ig)	6.3	1982-1992 (11)
35.5	KYOTO	25.4	1989-1993 (5)
35.5	TOTTORI	38.5	1981-1991 (11)
33.7	FUKUOKA	86.2	1991 (1)
32.5	NAGASAKI	26.7	1990-1993 (4)
31.5	KAGOSHIMA	1.0	1990 (1)

* not corrected by gear efficiencies

Settling season of juveniles

Although available data are limited, the settling date validated by otolith microstructures showed clear latitudinal differences: April-May in northern Kyushu (Shijiki Bay), May-June in the central Sea of Japan (Wakasa Bay), and June-July in the northern Sea of Japan (Igarashi-hama) (Figure 3, Tanaka et al.[4]).

Juvenile growth

Daily growth rates of the juveniles (30mm-60mm SL) estimated from modal shift of size composition varied with location (Figure 4, Tanaka et al.[3]). They correlated

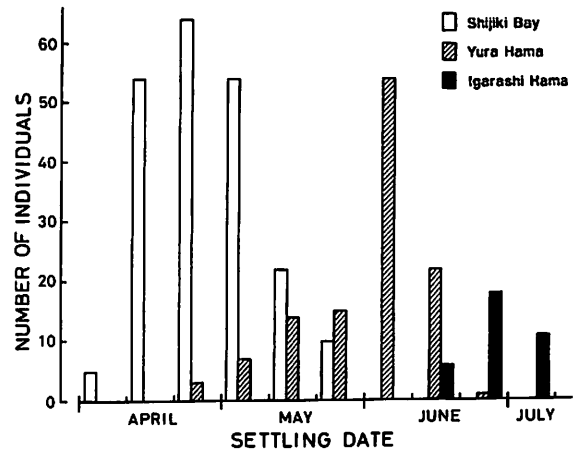

Figure 3. Settling-date distribution of the Japanese flounder in 10 day intervals estimated from otolith micro-structures (Tanaka et al.[4]).

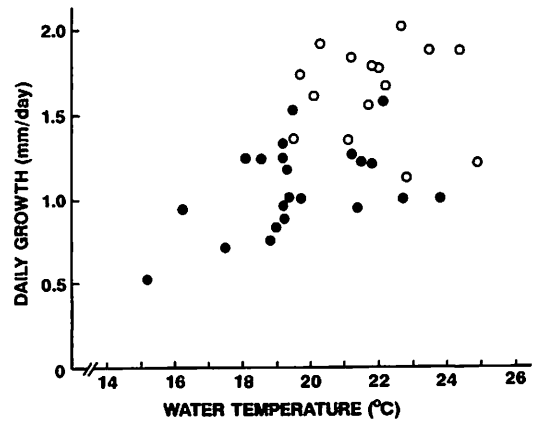

Figure 4. Daily growth rates (mm/day) relative to water temperature of the Japanese flounder juveniles collected at various localities. Daily growth rates were estimated from the change in body length distribution between 30 and 60 mm SL. Water temperatures are means from nursery areas. Solid circles represent the rate from southern and open ones from northern nurseries divided by the Noto Peninsula (Tanaka et al.[3]).

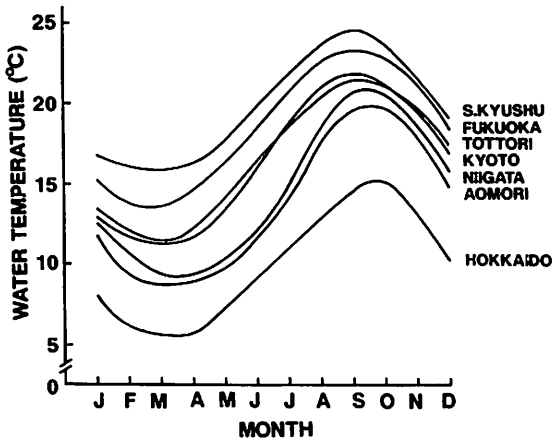

Figure 5. Seasonal changes in average water temperature at 50m depth at seven latitudinally different locations (Tanaka et al.[3]).

generally to average ambient water temperatures, but those obtained from northern regions were higher than those from the southern regions when compared for the same range of water temperature.

Juvenile abundance

Table 1 shows the average density of 0-group Japanese flounder at 10 latitudinally different nurseries. Although data accumulation (year) are considerably variable among the nurseries, a clear trend can be seen: northern low and southern high abundance except for southern range limit of Kagoshima (Tanaka et al.[3]).

Latitudinal differences in environmental variables

Water temperature

Seasonal changes in average water temperature at 50m depth show a latitudinal difference (Figure 5, Tanaka et al.[3]). When Hokkaido is compared to southern Kyushu, the temperature difference is approximately 10℃ throughout the year and the former highest temperature is still lower than the lowest temperature of the latter.

Prey animal abundance

Juvenile Japanese flounder have been described as a specialist feeder on mysid shrimp.[4,14-16] Average abundance of mysids during the settling and post-settling season is not so different among nurseries, most of them ranging between 100 and 300mg per m^2. However, the pattern of seasonal changes in abundance is different between the northern and southern nurseries. In southern areas, abundance decreases markedly when water temperature exceeds about 20 or 21℃, while it occurs at much higher water temperatures in Niigata and Yamagata and no significant reduction can be seen in the northernmost

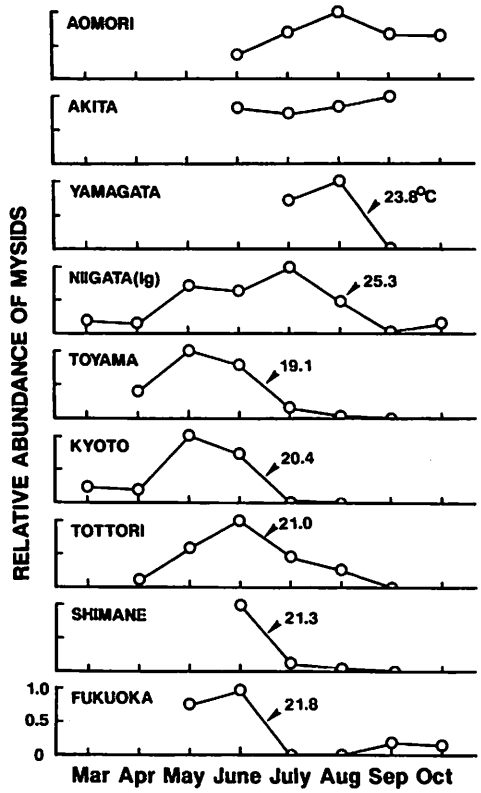

Figure 6. Seasonal changes in mysid abundance at nine latitudinally different nurseries. Water temperatures at time of decreasing abundance are shown. Abundance is ploted on a relative scale (Tanaka et al.[3]).

nurseries (Figure 6, Tanaka et al.[3]).

Predator abundance

Van der Veer and Bergman[17] demonstrated that brown shrimp *Crangon crangon* is a substantial predator for newly settled plaice *Pleuronectes platessa* juveniles in the western Wadden Sea. The Japanese brown shrimp *Crangon affinis* appears to be a predator for newly settled juveniles of stone flounder in Sendai Bay.[18] Although there is no direct field evidence of predation by crangonid shrimp on the Japanese flounder, experimental work[19] suggests that they act as a potential predator for the newly settled juveniles. The density of *Crangon* shows higher abundance in northern and lower in southern nurseries (Figure 7, Tanaka et al.[3]).

Geographical variations in meristic counts

Result from 1991 sampling

Four hundred juveniles which had been collected from 10 nurseries from Hokkaido to Fukuoka were examined for geographical variations in number of vertebrae, dorsal and anal fin rays. There was no significant variation in number of vertebrae, but a geographical variation was evident in the number of dorsal and anal fin-rays, particularly in

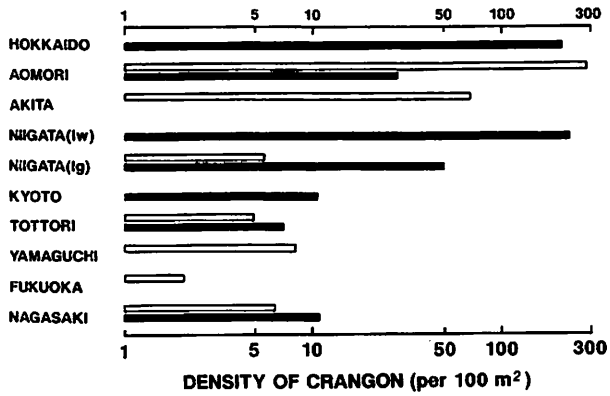

Figure 7. Average density of crangonid shrimps collected at 10 latitudinally different nurseries. Open and solid bars represent data from direct sampling in 1991 and data from literature, respectively. Dnsity is not corrected for gear efficiency (Tanaka et al.[3]).

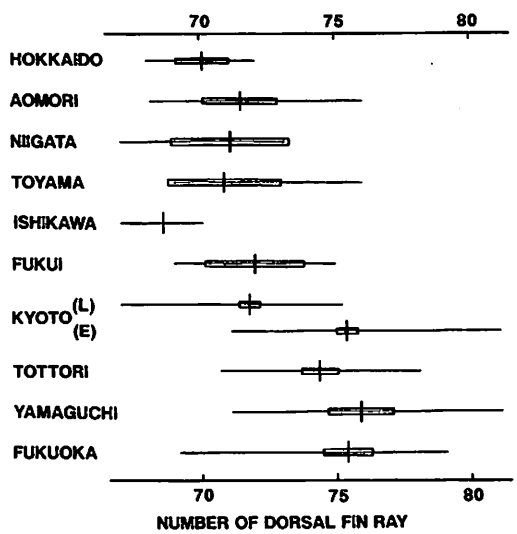

Figure 8. Geographic variation in number of dorsal fin-rays of Japanese flounder juveniles collected from 10 nurseries in 1991. Mean, standard deviation and range of the fin-ray number are shown for each nursery. Kyoto (E) and (L) indicate early and late settlers, respectively (Kinoshita et al.[5]).

dorsal fin (Figure 8, Kinoshita et al.[5]). Average number of dorsal fin-ray is around 71 and 72 in the northern nurseries, whereas it is around 75 in the southern nurseries. Kyoto located in the mid-coast of the Sea of Japan shows a seasonal difference in the count ; late settlers are lower and early settlers are higher in number.

Result from 1994 sampling

Dorsal fin-ray number of 1665 juveniles, collected from the Sea of Japan nurseries from southern Kagoshima to western Hokkaido, were reexamined to confirm the result of the 1991 sampling. Higher numbers in southern and

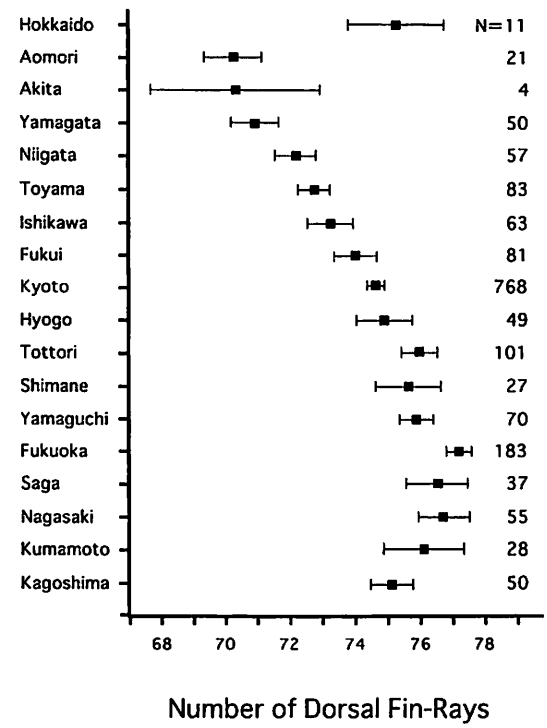

Figure 9. Geographic variation in number of dorsal fin-rays of the Japanese flounder juveniles collected from 18 nurseries (all prefectures) in 1994 (including a sample provided from Yamagata Prefectural Fisheries Experiment Station). Number of dorsal fin-rays is shown by mean and 95% confident limit (Tanaka et al.[6]).

lower in northern nurseries were confirmed again, but the geographical variations are continuous and there is no clear discontinuous boundary around Kyoto which was seen in the 1991 sampling (Figure 9, Tanaka et al.[6]). However, distribution of the fin-ray number at each nursery (Figure 10) shows there are very few juveniles of higher fin-ray number than 75 in northern areas beyond Toyama (except for Hokkaido), while there are few juveniles of lower fin-ray number than 75 in southern areas beyond Fukuoka.

Temperature effects on dorsal and anal fin-ray number

The number of meristic characters in fishes is fundamentally determined genetically, but considerable modification could occur under the influence of various environmental factors.[20] Among those factors, water temperature must predominantly influence the meristic counts. Experimental works on effects of rearing water temperature on the fin-ray number, which were done in 1991 and 1994, revealed that the number of dorsal and anal fin-rays increased with increases in ambient temperature (Figure 11, Seikai et al.[8]). A more clear result was obtained using cloned flounder larvae in 1995.

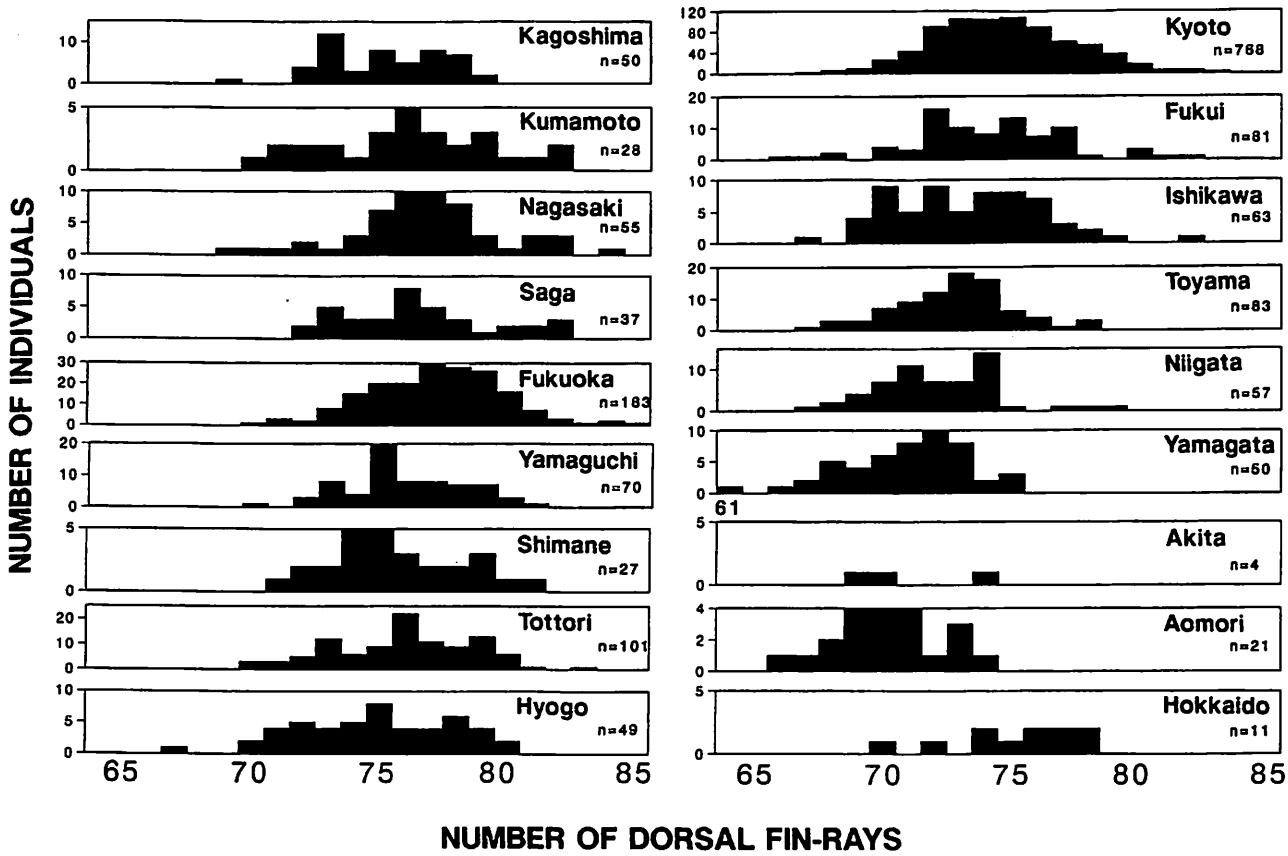

Figure 10. Distribution of dorsal fin-ray number of the Japanese flounder juveniles collected from 18 nurseries (data source and abbreviations as in Figure 9) (Tanaka et al.[6]).

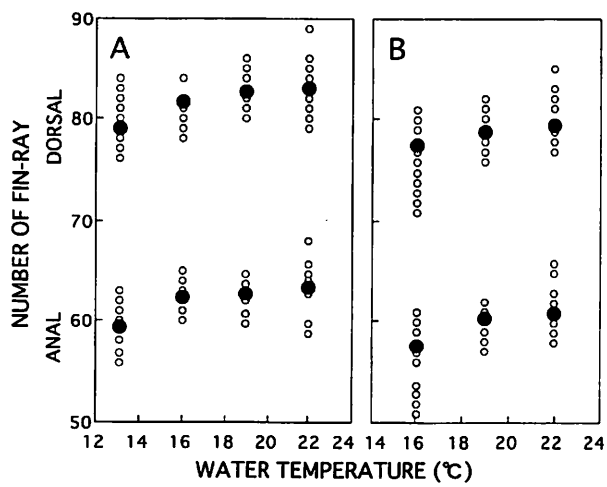

Figure 11. Expeimental results of rearing water temperatures on number of dorsal and anal fin-rays in the Japanese flounder. The laruae were kept at three or four different temperature regimes (A was done in 1991 and B in 1994) during the period from pre-flexion stage to post-metamorphic early juvenile stage(Seikai et al.[8]).

Analysis of the mitochondrial DNA

The first half of the control region in the mitochondrial DNA (mtDNA) was sequenced for two local populations from Niigata Prefecture.[9] The results showed that this region of the mtDNA is highly variable, and suggested that the two local populations differ somewhat genetically. A more extensive survey of variation in this mtDNA segment was made for several geographical populations throughout Japan, one from the Pacific coast and another from the Sea of Japan,[10] confirming the high sequence variability of this mtDNA region in the Japanese flounder. The sequence data from this study revealed that genetic make-up of these geographic populations are largely homogenous on the whole. Detailed examination of the data, however, indicated little but significant genetic differentiation among some geographical populations (Figure 12, Ohkawa et al.[10]).

DISCUSSION

The Japanese archipelago is primarily located in a temperate zone, but the southern islands and the northern island

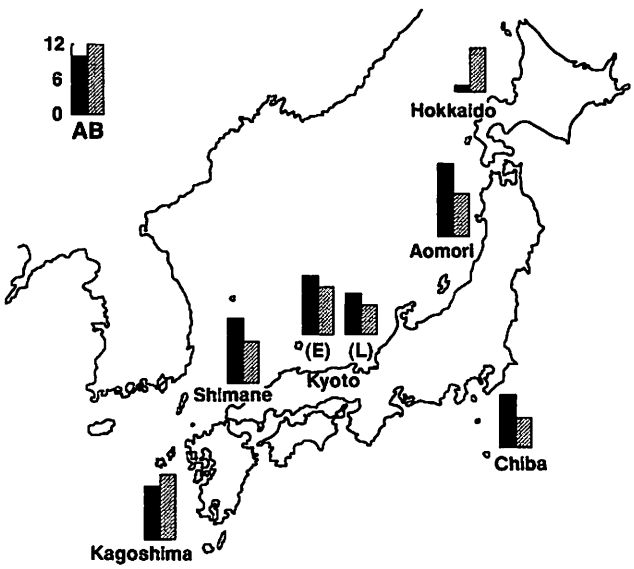

Figure 12. Proportion of haplotypes A and B of the Japanese flounder, which were identified from nucleotide-sequence data of mitochondrial DNA control region, at 5 Sea of Japan and one Pacific coastal areas (Ohkawa et al.[10]). Kyoto (E) and (L) represent early and late settlers, respectively.

are included in subtropical and subarctic zones, respectively. The Japanese flounder distributes around the Japanese archipelagos except Ryuku Islands and northern (Okhotsk coast) and eastern (Pacific coast) Hokkaido. Much higher abundance of the flounder can be seen in the coast of the Japan Sea compared with the Pacific coast. Although the Sea of Japan coast including the western coast of Kyushu is influenced by the Tsushima Warm Current, the physical environmental conditions are largely different depending on localities or latitudes. Water temperature, an important influence on growth and reproduction of fishes, is markedly different between the northern range limit of Hokkaido and the southern limit of Kagoshima as shown in Figure 5. The Japanese flounder would be expected to develop different survival strategies adapted to such extreme environments.

Various latitudinal variations were recognized in environmental conditions and life history traits of the flounder. Patterns of seasonal change in mysid abundance and potential predator (*Crangon*) abundance are apparent between northern and southern nurseries. Abundance of juvenile flounder and juvenile growth rate is also different between the north and south. There is a discontinuous shift of the main spawning season between Kyoto and Niigata with the Noto Penisula acting as a boundary dividing the north and south. Dorsal fin-ray analysis in 1991 clearly demonstrated a high number in the southern group and a low number in the northern group separated by the Noto Peninsula. If there were different stocks in the north and south, patterns of annual fluctuation in the juvenile abundance may be different between these two. A preliminary analysis[3] suggests this, but more detailed and long-term data accumulation and analysis are needed.

Dorsal fin-ray numbers showed a southern-higher and northern-lower trend in both 1991 and 1994 samples. From the result of 1994 samples we can expect that a gradual and continuous decrease in dorsal fin-ray numbers from Fukuoka to Aomori may be caused by gradients of some environmental variables. Water temperature as shown in Figure 5 particularly shows such a gradient with southern-higher and northern-lower. From Figure 2 showing a latitudinal difference in the spawning season and Figure 3 showing settling season, we can estimate the approximate water temperature during early ontogeny at the three nurseries. Seikai (unpublished) suggests that the critical developmental phases influencing the number of fin-rays are embryo formation stage and initiation stage of metamorphosis. Estimated water temperatures are about 13-16, 12-16, and 15-18, 17-20℃ in Shijiki Bay, Wakasa Bay, and Igarashi (Niigata), respectively. Thus, water temperatures experienced by early ontogenetic flounder (eggs and larvae) are rather high in northern nurseries.

The experimental work testing effect of water temperature on dorsal fin-ray numbers[8] demonstrated that lower temperature resulted in lower number of the fin-ray. Higher number of dorsal fin-rays is characteristic of flounder from southern nurseries where the larvae settle at relatively lower temperature. These findings strongly suggest that geographic differences in fin-ray numbers are not caused by water temperature after birth but are under genetic control.

The largest discrepancy in the results of meristic counts between 1991 and 1994 is that the discontinuous difference in the fin-ray number between the north and south found in 1991 is not clear in 1994 samples. This can presumably be attributed to a large difference in sample size, 1994 being four times that of 1991. Kinoshita et al.[5] demonstrated that two different populations settled in Kyoto; early settlers have higher numbers and later settlers lower numbers of the fin-ray. The same phenomenon can be seen in adjacent prefectures as Hyogo, Fukui and Ishikawa. If there are two different stocks they would be expected to share boundary areas as habitats as the relatively large southern stock may recruit into the northern habitat by transport during the pelagic larval period by the Tsushima Current. When we increased the sample size as done in 1994, average dorsal fin-ray number decreases toward the north. This may reflect a decreasing proportion of the southern stock in progressively northern nursery areas.

The mitochondrial DNA has been known as a useful tool for analyzing stock structure (e.g. Pitch and Calbalko[21]). Actually, the control region of this DNA in the Japanese flounder was found to be highly variable. Squence data so far obtained from this region of mtDNA indicated little but significant genetic differentiation among geographical[10] as

well as local populations[9] around Japan. This finding provides some support for the notion of existence of stock structuring in this species. The basic homogeneity observed, however, suggests that the origin of the structuring may have occurred rather recently, or ample gene flow may have maintained among the structured stocks.

The Sea of Japan was connected to the East China Sea through the Tsushima Strait and to the Pacific Ocean through the Tsugaru Strait when the sea level rose to the present level about 10 thousand years ago. It seems reasonable to speculate that different stocks, originally distributed in the southern Pacific and northern Pacific colonized in northwestern Kyushu and south-western Hokkaido, respectively. Based on evidences and preliminary consideration, we could hypothesize four different stocks in the Sea of Japan : northwestern Kyushu, southern Sea of Japan, northern Sea of Japan and western Hokkaido. This hypothesized stock structure should be carefully tested using reproductive and early ontogenetic ecology and other potential molecular genetic analyses.

Although the genetic background of stock structure is still insufficiently understood, we should carefully consider geographic ecological differences and the possible existence of local stocks as sea-farming, using mass-releases, proceeds. Recent advances in biotechnology for the flounder have revealed that water temperature significantly influences sexual differentiation-higher temperature resulting in a high percentage of males.[22,23] Tabata (personal communication) suggests that the critical water temperature for this effect may differ between northern and southern stocks. This may be true of the temperature effect on meristic counts also. Careful ecological and physiological research should be conducted to determine the effects of temperature relative to development of seedling production and release strategy. Clearly, potential differences in the performance of different stocks relative to such environmental factors as temperature can have a dramatic impact on immediate and long term impacts of sea farming.

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AN ECOPHYSIOLOGICAL MODEL FOR PREDICTING PERFORMANCE OF RELEASED FISH

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ABSTRACT

By extending an ecophysiological model at the metabolic level to the level of the subpopulation, we derive a method for estimating productive capacity of habitats. The model requires input from lower levels in the form of scope for production, which, along with stock size, can estimate marginal scope for production. Scope for production is a function of intrinsic capacity of subpopulations to grow and environmental factors. Marginal scope for production is the increment of biomass per unit habitat and thus an estimator of habitat quality. An application of this model is described whereby optimal biomass of released fish into nursery areas can be estimated.

INTRODUCTION

The optimum biomass of released juvenile fish depends upon the productivity, or capacity of the habitat to support production, including that of the same, or competing, species colonizing that habitat. Specifically, the optimum biomass for stocking is the difference between that which fully utilizes the productive capacity and the wild biomass. Releasing less means wasted production; overstocking usually means mortality, emigration or slow growth, i.e., density-dependent regulation downwards.[1)]

Ability of habitat to support production is often estimated as carrying capacity, which refers to the maximum biomass (B_{cc}) the system can support with no net growth. Ideally, the carrying capacity should be reached at the end of the period of occupancy, for example, at the end of the juvenile season, before emigration occurs to the adult habitat. The ideal biomass at the beginning of the season is that which will grow to the carrying capacity at the end of the season, that is :

$$B_{released} = B_{opt} - B_{wild},$$

where Bopt is that biomass where $B^{(G-Z)} = B_{cc}$ at the end of the period.

Experimental releases to determine the carrying capacity is expensive in terms of time and numbers of hatchery fish. In principle, if one could estimate the productive capacity of a habitat, it should be proportional to carrying capacity. The productive capacity, or quality, of a habitat is a complex function of interacting environmental factors[2)] and is stage- and species- specific. What follows is a model that integrates the effects of multiple, time-varying, abiotic factors on the performance of fish. The habitat is that of juvenile nursery areas. Being stage- and location-specific, the appropriate ecological level is that of the subpopulation.

ECOPHYSIOLOGICAL FRAMEWORK

Fry[3)] developed a scheme for integrating the effects of environmental factors on fish metabolism, which is the "engine" that enables fish to perform all activity, as well as survive. Activity, in Fry's terminology, included swimming, feeding, digestion, growth, and indeed, all activities in support of life. Much knowledge existed on effects of single factors, e.g., temperature, on single activities of fish, e.g., growth, but no one knew how to integrate multiple factors, other than simple addition -- which was unsatisfactory, at least outside the range of observed values. Fry's genius was to consider how factors affected metabolic capacity and classify factors based on mechanisms.

Fry[3)] suggested the metabolic energy available for activity was the difference between the maximum, or active, rate and that necessary for maintenance, or standard metabolism. This difference he called metabolic scope, which varied in response to the fish's environment, and is equivalent to metabolic capacity. Fry found 5 distinct classes of factor effects on metabolism, which, in turn, set the limits of fish activity. In general, environmental factors operated on scope either by affecting active or standard metabolism, or by altering the internal state of the fish.

> *Controlling* factors (temperature, pH, pressure, et al.) set the pace of both maximum and maintenance metabolism ;

Limiting factors (oxygen, micronutrients, certain metabolites and pollutants that interfere with oxygen uptake, et al.) constrain the maximum rate
Masking factors (sub-optimal salinity, disease, certain pollutants and environmental stressors, et al.) increase, or "load", standard, or maintenance, metabolism, by increasing obligatory metabolic work;
Directive factors either "unload" standard or "release" active metabolism by putting fish in a microhabitat or physiological state where it is better (pre) adapted (temperature, light, photoperiod, et al.), that is, they cause distributional, acclimatory or anticipatory responses. Directive factors generally involve feedback between fish and environment, others do not; and,
Lethal factors (toxins, supersaturated gases, acute lethal temperatures, et al.) interdict metabolism to cause death. Death can also occur if a fish's metabolic scope falls below the maintenance level for too long.

Any factor, such as temperature, may function as more than one factor type, and cause multiple responses of fish, including directive responses, according to species, stage, and acclimation state. This fundamental realization formed the basis of his metabolic model, which has proved remarkably robust over the years.

METABOLIC MODEL :RED DRUM AND FLOUNDER

Building on Fry's framework we developed, and calibrated, a model for juvenile red drum (*Sciaenops ocellatus*, Sciaenidae) which predicted metabolism within 20% in an aquaculture setting. By far, the most important factors affecting red drum metabolic scope were oxygen (a limiting factor) and temperature (a controlling factor). Salinity (a masking factor) had little effect. Directive and lethal factors were not particularly relevant in the aquaculture setting.

In estuarine or coastal nursery areas, the environment of juvenile fish is characterized by diel or tidal cycles of most environmental variables. In more isolated nurseries, such as embayments of Pamlico Sound, NC, USA, environmental variability is less predictable, being driven by meteorology and seasonal changes. Juvenile fish in either system may be regularly exposed to lethally high temperatures or low dissolved oxygen levels in late summer and must migrate in and out of marsh creeks to optimize environmental conditions. Directive factors are of paramount importance in such systems. Consistent with the aquaculture findings, dissolved oxygen appears to key most fish movements in and out of these systems (J. Lancaster, NCSU, unpubl. data).

This summer, caged juvenile summer flounder (*Paralichthys dentatus*) in these creeks, were used to field test of our flatfish metabolic model. Preliminary analysis of the data showed the metabolic scope of juvenile summer flounder at 4 sites is, at least ordinally, predicted correctly by our model (K. Duchon, NCSU, unpubl. data).

But the performance of released fish we usually are interested in is production, that is, survival and growth. But before moving on to the subpopulation level, let's summarize. Flatfish behave generically like red drum; that is, the model worked with a little adjustment of rates. Second, in NC estuarine nursery areas, directive factors are of paramount importance. Red drum juveniles must migrate in and out with the tide to utilize any of the production in the marsh *in situ*, or to gain any supposed refuge from predators. Juvenile flatfish, on the other hand, appear to be better adapted to withstand temporarily sub-optimal environmental conditions . Although we need to do more work on directive factors, including behavior, we now have a framework to design experiments and interpret results.

MODEL APPLICATION AT HIGHER LEVELS

Building on Fry's scheme, we[4] suggested a framework for understanding interannual variability in flatfish recruitment. In that work, we suggested analogs of Fry's metabolic factor types at the population, and higher, levels of biological organization. Recruitment is a population-level response, and while the ultimate aim of stock enhancement is to rehabilitate populations, we begin with a subpopulation model of juveniles in their nursery areas. The model correctly predicted metabolism and growth, an individual-level response, in an aquaculture setting. To predict subpopulation growth (production) we must define factor types for at least three levels of organization -- metabolic, individual, and subpopulation. Figure 1 shows example factor types at four levels, along with their corresponding scopes. The general hypothesis is that individual, subpopulation and population scopes for growth, subpopulation growth (or production) and recruitment to the population, respectively, respond to controlling, limiting and masking factors in an analogous way to the metabolic scope response Fry[3] found.

PERFORMANCE MODEL : SCOPE FOR INDIVIDUAL GROWTH

The generic scope model suggested a way to predict performance. Metabolic scope is what supports growth, and scope for growth supports production by a subpopulation. But metabolic scope is at a level below that of an individual, so we had to develop a conceptual method of moving scope between levels. Since growth is a product of

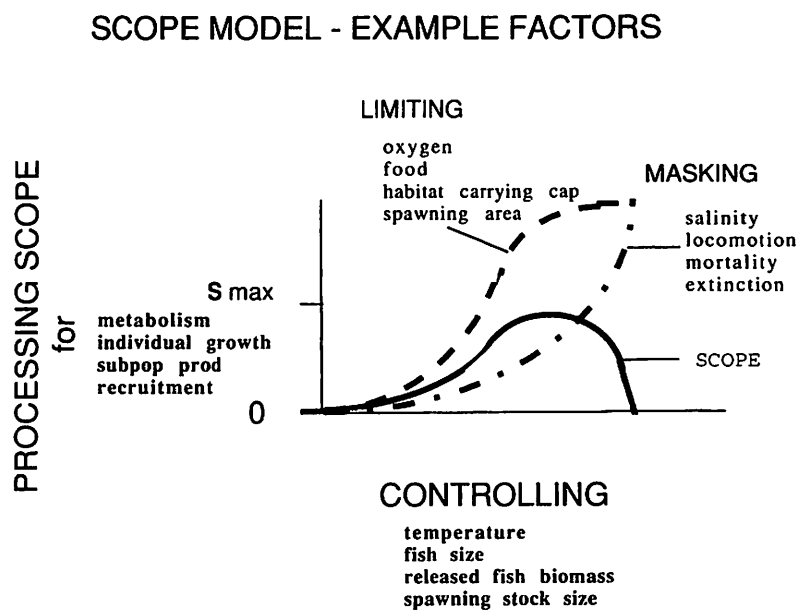

Figure 1. Scope model and example factors at 4 levels of organization: metabolic, individual, subpopulation and population. Limiting factors affect the maximum metabolic rate; masking factors affect the maintenance metabolic rate. The difference between maximum and maintenance rates is scope for processing.

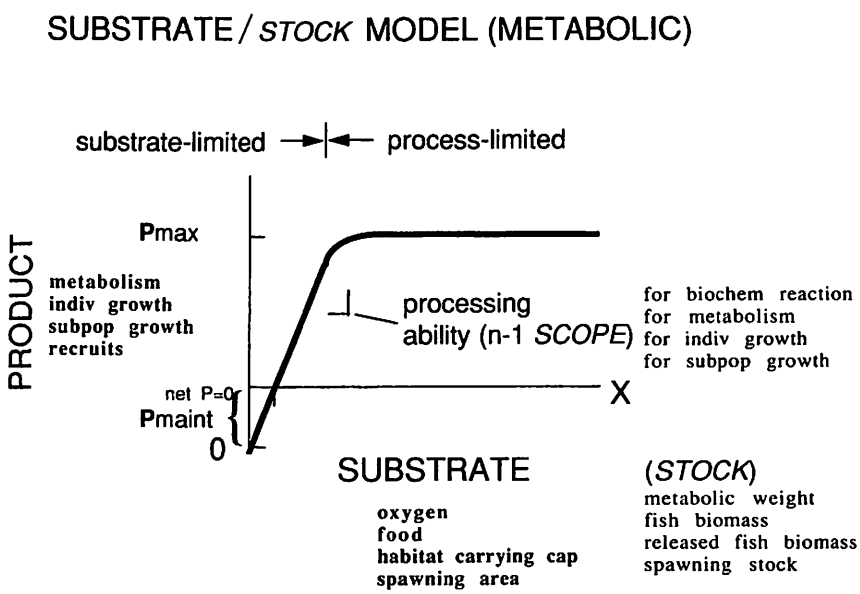

Figure 2. Product vs. substrate model showing processing ability is the scope at n-1 level X the stock for 4 levels of organization. Pmaint is the amount of production to sustain the stock; net P = 0 where P = P_{maint} ; P_{max} is the maximum rate of production when stock or biomass is limiting, i.e., above the limiting substrate concentration.

the metabolic "engine", its ability to process substrate (food) should be a function of its state, which is another way of expressing capability, that is, scope. Thus, metabolic scope becomes a state variable at the individual (next highest) level (Figure 2). Substrate is processed into product at a rate dependent upon state (scope at n-1 level) and stock. Scope at the next highest level is thus analogous to "intrinsic rate of increase" or the half-saturation K in Michaelis-Menten kinetics. In our scheme, the integrated effects of environment, that is, scope, becomes a controlling

factor at the next highest level. Figure 2 shows the corresponding substrates, stocks and n-1 scopes for the four levels of biological organization. Also shown on Figure. 2 is the maximum production (Pmax) and the maintenance production (Pmaint). The former is the upper limit of production dictated by the particular stock and its state, if substrate is not limiting. Pmaint is equivalent to the rate of production necessary to sustain the stock at 0 net production. Up to the substrate level which saturates the stock, production is limited by substrate. At higher levels of substrate, stock processing ability is limiting. The example in Figure 2 shows how environment affects individual growth. First, the effects of environment are integrated into metabolic scope, which becomes a controlling factor, and second, additional environmental effects occur at the individual level, which in turn, becomes scope for individual growth. Individual scope for growth becomes a controlling factor at the subpopulation level, and subpopulation scope is processing ability at the population level. Scope, thus conceived, is also why typical stock-recruitment curves may not work, since most consider stock size to be the only independent (controlling) variable.

PERFORMANCE MODEL : MARGINAL SCOPE FOR PRODUCTION

But how do we estimate carrying capacity, or Pmax, without conducting a series of subpopulation releases? Neill and Bryan[5] defined marginal metabolic scope and suggested it could be used to estimate maximum metabolism. Consider a fish metabolizing at any routine rate (RMR) while oxygen declines. The fish, by compensatory increases in breathing, stroke-volume, et al., can support the routine rate over some range of dissolved oxygen (a substrate), i.e., its respiration rate is independent of oxygen. However, at some level of the declining oxygen, called the limiting oxygen concentration (LOC), the fish can no longer compensate, and its metabolic rate becomes oxygen-dependent. The longer the fish is able to compensate, that is, the lower the limiting oxygen concentration (LOC) for that rate, the more energy reserves, or more accurately, the more reserve power[6] the fish had. This reserve power is exactly metabolic scope. Thus Neill and Bryan[5] showed the derivative of metabolic scope, as it went to 0 at the LOC can be estimated, via respirometry, from the ratio of RMR to LOC. (Actually, they used LOC +1 unit of oxygen to unitize the ratio). The analogous ratios at higher levels represent the gain in product per unit substrate, and is equivalent to the state, or processing ability, of the stock in Figure 2.

The concept of marginal metabolic scope can also be extended to marginal scope for subpopulation growth (Figure 3), and thus can be estimated via a series of manipulations of substrate / stock, in this case, habitat/ biomass of fish. The ratio of production to the limiting habitat is equivalent to RMR / LOC, and can be estimated by enclosing equivalent biomasses of juvenile fish in different sized enclosures.[7] Alternatively, biomass could be manipulated in enclosures of equivalent size. As long as range of the available substrate (habitat) includes the

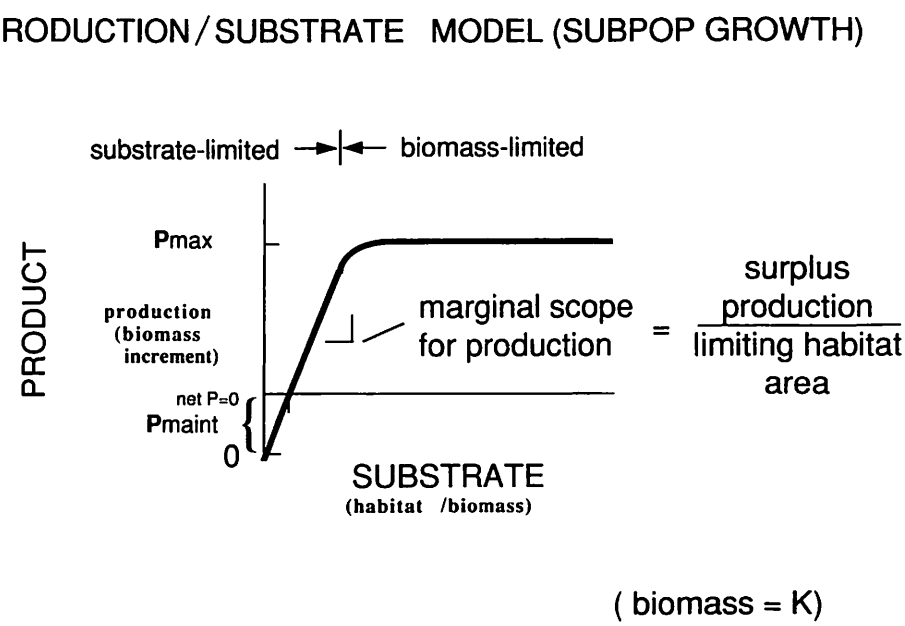

Figure 3. Production vs. substrate (habitat area) for subpopulation growth where biomass of stock is constant. Marginal scope for production is the rate of increase in product per unit substrate, and is estimated by the ratio of surplus (net) production to the limiting habitat area for that stock.

substrate for Pmax for that biomass, the limiting substrate / biomass can be determined for a given level of production. The ratio of limiting level of substrate to production is the rate of increase in P per unit substrate, or scope for production. In principle, this is proportional to habitat quality. From this the optimum biomass for a nursery area can be determined, and thus the optimum biomass for stocking if the wild biomass is known.

CONCLUSION AND PROGRESS

This paper has outlined a model and a methodology for determining the capacity for production in nursery areas. As such it is also a method for determining habitat quality in a meaningful way, marginal production per unit habitat. A practical application would be to estimate optimal stocking density for a nursery. However, to understand, that is to predict outside the range of observation, nothing less than a model built from the metabolic level up, beginning with a knowledge of how scope responds to environmental factors at least 2 levels, n and n-1, is likely to suffice. Collecting synoptic environmental data on physiologically- and ecologically-relevant scales is critical to understand how environment operates at the metabolic, and higher, levels. It matters not what the average daytime dissolved oxygen level is, if it falls below a lethal level for a few minutes. And, the effects of one factor, e.g., oxygen, cannot be interpreted without synoptic measurements of other factors.

The subpopulation model for released juvenile flatfish we are refining is internally consistent, and experimental releases will follow this spring in Japan and the US. It seems most likely that the main determinants of performance will be carrying capacity (limiting at the subpopulation level), food (limiting at the individual level) and fish distribution within the nursery (response to directive factors at the subpopulation level). We also expect the metabolic (and, therefore, growth) scope of fish, as well as the relative importance of carrying capacity (limiting) and predation (masking) to vary within the range[8)] and as a function of timing of release. A test of understanding will be our ability to predict performance in different (than experimental) nursery areas on the basis of quantification of key factors in advance of releases. Once we understand the scope for performance, then impacts of stocking on nursery ecosystems can be estimated, as well as the optimum biomass for stocking.

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QUALITY OF FISH FOR RELEASE: BEHAVIORAL APPROACH

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ABSTRACT

Studies on the quality of fish are reviewed to describe the present status and the future direction of this field. To be healthy and to have the same function and ability as wild fish are fundamental and necessary conditions for the juveniles for release. These conditions, however, are not always sufficient, since the seedlings are required to have adequate behavioral characteristics which enable them to adapt to the natural environment after release. Quality of fish should be estimated by using an index which directly reflects stocking effectiveness, such as survival and growth in the field. Morphological and physiological characters such as body color, swimming ability, osmoregulatory function or some enzyme activities are often studied. However, these are sometimes indirect, while appropriate behavioral characteristics are sensitive to express the adaptability to the natural environment. Jumping behavior in the ayu, *Plecoglossus altivelis*, is a predictive index for the stocking effectiveness which is closely related to upstream swimming just after release and thus recapture rate, while tilting behavior in the red sea bream, *Pagrus major*, represents a tendency of cautiousness which may reduce the risk of predation. In reared juveniles of Japanese flounder, *Paralichthys olivaceus*, longer duration of off-bottom swimming during feeding behavior are most vulnerable to attack by predators, especially by older individuals of conspecies. Such behavioral characteristics are not only potentially applicable to improvement of the seed production technique but also can be an indices to measure the quality of fish for release.

INTRODUCTION

Stock enhancement or propagation is fundamentally different from aquaculture in many points although both are carried out in water.[1] The former aims to increase the number of individuals through reproduction as well as body size in the natural environment without artificial feeding, while the latter expects the increase of only body size through intensive feeding in a restricted pen cage or aquarium. The characteristics of these two categories might correspond to those of fisheries as hunting in water and planned agriculture on earth, respectively. Furthermore, the disciplines of science which support each field are also different: i. e. aquaculture is mainly concerned with physiology while propagation with ecology. Although the research on aquaculture has made remarkable progress recently, fundamental research is scarce in propagation.

Many problems around stock enhancement have been pointed out such as scattering of pathogens, genetic pollution, ecological disturbance, etc.[2-4] Fishery production in the coastal waters, on the other hand, have not shown a significant increase in two major marine stock enhancement species in Japan-- red sea bream, *Pagrus major*, and Japanese flounder, *Paralichthys olivaceus*-- in spite of an expanding number of seedlings released in the past 20 years.[5] One of the reasons for this unsatisfying outcome might be that we have only limited data on the effect of stock enhancement in the natural environment and the interactions to wild conspecies or other species. Therefore, the first priority must be to understand the process of adaptation of released fish to the natural environment and to estimate the effectiveness of stock enhancement quantitatively using both scientific and economic measures.

Stocking effectiveness is determined by the following three factors[6]: (1) releasing technique as an issue of man, (2) quality of fish as an issue of seedlings, and (3) the environmental condition as an issue of the field for stock enhancement (Figure 1). We have substantial knowledge on the releasing technique while little is known about the

Factors Affecting Stocking Effectiveness

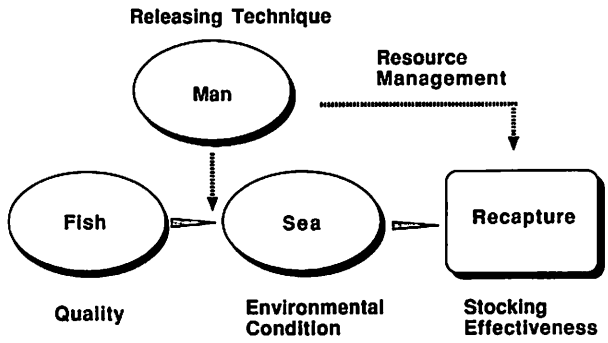

Figure 1. Three factors which determine the stocking effectiveness of released fish.[6]

fish quality and the environmental condition. Although it may be difficult to improve the environmental condition, we can improve the fish quality of seedlings and expect the increase in stocking effectiveness.

In this paper, we mainly deal with fish quality in relation to stocking effectiveness. First, we describe (1) the fundamental concept of stock enhancement, and present (2) the example of behavioral research on fish quality. We also discuss (3) the importance of the behavioral index for the research of fish quality and stock enhancement.

FUNDAMENTAL CONCEPT OF STOCK ENHANCEMENT

Stock enhancement should be based on the concept of the ecosystem. We usually release the seedlings of species which are economically important and few in number. These species belong to a relatively higher class in the ecological pyramid. Since the production of a class is determined by that of one lower class in the pyramid, we cannot release seedlings exceeding the capacity of the lower class.

The present ecological pyramid may be deformed by an intensive fishing pressure and this tendency may be more severe especially in the upper classes of the pyramid. Severe exploitation of marine biological resources for this half century may yield a vacant niche or an unutilized capacity near the top of the pyramid. The fundamental concept of stock enhancement is to use this unutilized capacity by releasing the seedlings into a vacant niche of the pyramid. However, we have little information about how much the unutilized capacity exists in the ecosystem, how many seedlings we should release to the field to make up the deformed pyramid, and how we can manage an effective and sustainable utilization of the productivity in nature. An ecological survey of the stocking area is therefore indispensable in each stock enhancement project.

JUMPING BEHAVIOR OF AYU

Pilot release of artificially reared juveniles of the ayu, *Plecoglossus altivelis*, an amphidromous Salmonoidei fish with only a one-year life span, has been carried out extensively in Japan since the 1970s. However, almost all of the trials were unsuccessful. Experimental release in the Tsubusa River in Oita Prefecture revealed that the main cause of low recapture rate of released fish was the rapid downstream migration just after release and disappearance from the stocking area.[7] Wild juveniles released in the same river migrated upstream after release and showed a high recapture rate. Thus, in the stocking of ayu, the effectiveness represented by recapture rate in the stocked area was closely related to the tendency to swim upstream in the river.[8]

Juveniles of ayu show vigorous upstream migration in spring. They show active jumping behavior when they are confronted with waterfall. Since jumping behavior seems to be an element of upstream migratory behavior, the relationship of jumping behavior and tendency to migrate upstream was examined.[8] As a result, we confirmed that jumping activity measured in the laboratory was positively correlated with the tendency to swim upstream in a river after release. Jumping behavior was quantitatively measured by a simple apparatus (Figure 2) and jumping rate was obtained as a number of jumps a unit time. Many factors had potential to affect the jumping activity; i.e. high water temperature, change in light intensity, high fish density, hunger, and shallow water depth, all of which facilitated the jumping behavior.[9] Different stocks or fish groups of ayu were compared under fixed conditions with a relative strength of this behavior to a reference of the landlocked fish in Lake Biwa which showed a constantly high activity.[8,10] Jumping behavior was concluded to be used as a behavioral index to predict the

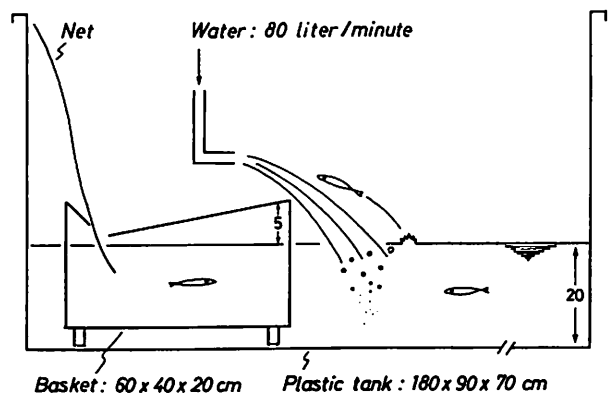

Figure 2. Apparatus used for the measurement of jumping activity of ayu, *Plecoglossus altivelis*.[8,10]

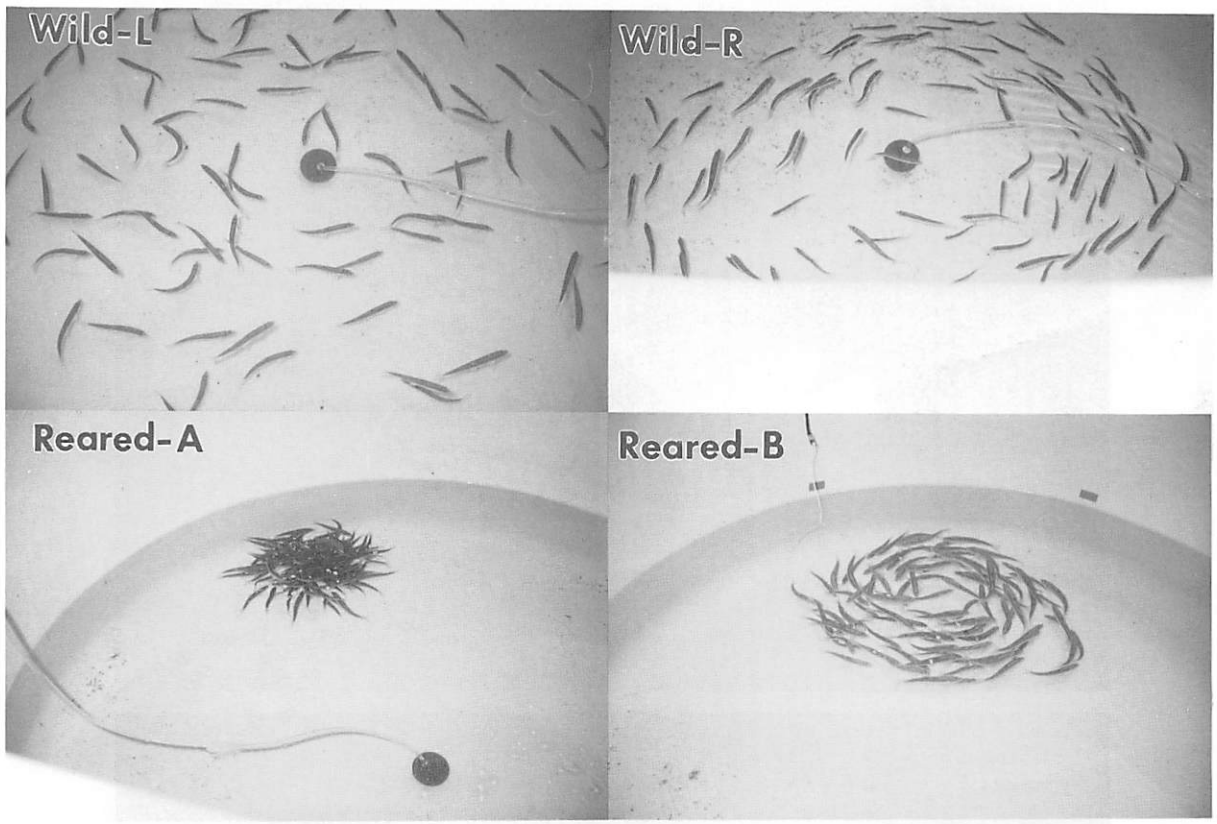

Figure 3. Typical spacing behavior of the juvenile ayu, *Plecoglossus altivelis*, of four different stocks (groups) in the daytime (standard length; ca 77 mm) : landlocked stock from the Lake Biwa (Wild-L) in "dispersion", amphidromous stock from the River Yabe (Wild-R) in loose "schooling", and two groups of reared fish from Hatchery A and B (Reared-A and Reared-B) in "pod" and tight "schooling", respectively.[10]

tendency to swim upstream after release and thus the recapture rate.

Another behavioral index studied in the ayu was spacing behavior (Figure 3).[10] Fish which presented a tight aggregation (or 'pod') in aquarium (Reared-A in Figure 3) showed a low activity of jumping, while those (Wild-L) with loose aggregation (or rather 'dispersion') jumped actively. Intermediate fish groups such as Reared-B and Wild-R in Figure 3 showed intermediate values of jumping activity. The difference in spacing behavior was the difference in the interindividual distance which was assumed to be caused by the difference in the "repulsion" between individuals. Thus, it might be concluded that fish with strong repulsion showed a loose spacing, high jumping activity, strong tendency to swim upstream, and high recapture rate or high stocking effectiveness, and vice versa.

TILTING BEHAVIOR OF RED SEA BREAM ——

In the case of the red sea bream, tilting behavior was validated as an index which represented the stocking effectiveness in the field (Figure 4).[11] This behavior was often observed when fish, irrespective of wild or reared, were caught and transferred from the natural field or a large net cage to an abnormal and unfamiliar circumstance such as a narrow bucket or a dazzling container. Fish aggregated tilting their body on the bottom with stretched dorsal fin, remarkable black stripes and fine cobalt blue spots on the lateral side. They stayed at one place, while moving their eyeballs frequently.[11] This behavior was considered to be a fear response to a frightening stimulus from the outside, and could represent the degree of cautiousness of the individual. The individual which was more cautious to a stimulus showed a tilting behavior for a longer time. The tendency of this behavior was concluded as a kind of individuality from the continuity of the trait or tendency in a series of repeated experiments using 85 marked individuals. Therefore we could quantify the cautiousness of the individual by measuring the duration of tilting behavior.

Through the stocking experiment in the field, the majority of post-stocking mortality occurred mainly because of predation by other fishes or larger conspecific just after release, on the day or the next day of release.[12] Laboratory experiments revealed that non-tilting fish were highly vulnerable to predation compared to the tilting ones

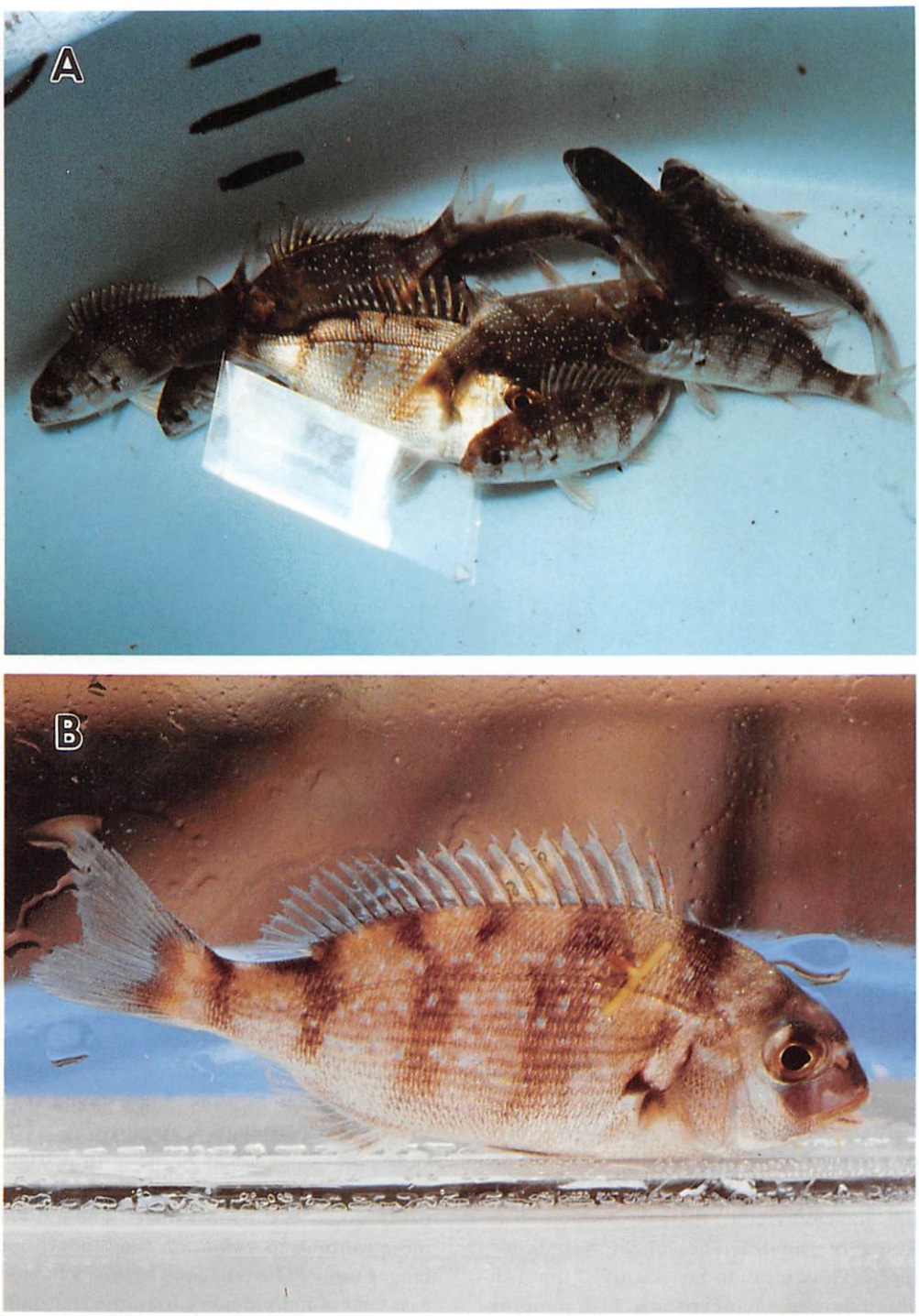

Figure 4. Tilting behavior of the red sea bream, *Pagrus major*.[11] A: Fish 40 mm in total length immediately after being released in the experimental tank (50 cm in diameter, filled to 30 cm with water) aggregating and exhibiting tilting behavior. B: A fish (83 mm TL) in a transparent photo-tank. Tilting behavior is characterized as tilting the body on the bottom, stretching their fins, and exhibiting cobalt blue spots and six dark stripes on their heads and bodies. This behavior is a fear response to such frightening stimuli as release, handling, and visual contact with humans.

Table 1. Relationship between tendency to exhibit tilting behavior and predation or recapture rate in the red sea bream, *Pagrus major* (data from Uchida et al.[11]). Laboratory experiment was conducted to estimate the vulnerability to predator (3-year-old red sea bream; 340-390 mm TL) in tilting group (30 fish; 80 mm TL) and non-tilting group (30 fish; 77 mm TL). Field stocking experiment was also carried out to compare the recapture rate of tilting (252 fish; 127 mm TL) and non-tilting fish (518 fish; 130mm TL) around the release point.

Fish	Predation (%)	Recapture (%)
Tilt	0	15.1
Non-Tilt	16.7	8.9

(Table 1). Field stocking experiments also confirmed that tilting fish stayed near the point of release with higher recapture rate while non-tilting fish dispersed after release. These facts showed that tilting behavior could represent the post-stocking mortality and thus, was available as a predictive index for stocking effectiveness of this species.

BEHAVIORAL QUALITY IN OTHER SPECIES

In masu salmon *Oncorhynchus masou*, contrary to ayu, wild stock showed stronger schooling behavior in earlier stages compared to the reared ones.[13] Schooling behavior is supposed to play an important role in their seaward migration, and this behavioral characteristic might be weakened by domestication.

Japanese flounder juveniles are considered to be vulnerable to predation during their off-bottom behavior while feeding, the duration of which takes longer in artificial seedlings compared to the wild ones.[14,15] This behavioral deficiency can be improved by acclimation to the natural field.[14] High mortality in released flounder might also happen at night, since, in the laboratory experiments, they show strong nocturnal behavior, but do not bury into sand well.[16]

Behavioral quality is also applicable to improve the releasing technique. In striped jack marine ranching, fish under strong stress tend to show spiral diving behavior and migrate away from the released site immediately after release.[17] This diving behavior, measured by maximum swimming depth within 10 minutes after release, is mitigated by gentle release, acclimation to the releasing area, and the training to the platform.[18]

FISH QUALITY AND BEHAVIORAL INDEX

Fish quality is defined as an aptitude for release: how many fish survive in the field after release and how much they yield to stocking effectiveness (Figure 5). In the study of fish quality, we have paid much attention to physiological and morphological problems of the seedlings but much less to the behavioral aspect. To solve both physiological and morphological problems should be the fundamental condition for the quality of fish: that is, health must be the prerequisite of the seedlings for release. However, being healthy does not always satisfy the quality of fish which directly connects to the stocking effectiveness represented by recapture rate (Table 2). Besides physiology and morphology, behavior of seedlings should be involved in the category of fish quality-- all these factors form the necessary sufficient conditions for fish quality. Recent techniques of mass production of seedlings have made much improvement and we can now produce many healthy fish which are almost equivalent to wild counterparts. However, behavioral problems of the seedlings have remained unsolved. Furthermore, behavior is most closely related to the ecological problems in the natural field, where they must be adapted and their survival and growth, and finally stocking effectiveness, are thus determined. Therefore we emphasize the importance of behavioral aspects in the study of fish quality.

We summarize behavioral indices of several species studied and their main causes of post-stocking mortality (Table 3). It is important to find suitable behavioral characteristics for each species which link directly to the cause of post-stocking mortality or reflect the stocking effectiveness. Also it is important to understand the meaning of the psychological drive which lies in the background of each behavior.

Through the technique of mass production, we can improve the behavior of the artificial seedlings to be equivalent to that of the wild (Figure 6), since behavioral

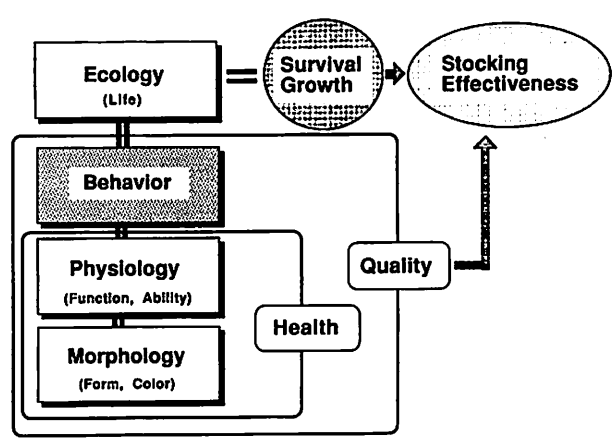

Figure 5. Diagram showing the interrelationship between stocking effectiveness represented by post-stocking survival/growth and four different disciplines of science which are closely related to biology of stock enhancement. Categories of fish quality and healthiness are also defined.[6]

Table 2. Swimming ability and stocking effectiveness in ayu, *Plecoglossus altivelis*. Swimming ability of five different groups of juvenile ayu was measured in laboratory and then released in a river to compare the recapture rate (data from Tsukamoto et al.[8]).

Fish group	Standard length*1 (cm)	Swimming ability*2	Recapture rate (%)
A	6.2±0.5	1639	35.3
B	7.6±0.7	1610	25.2
C	6.5±0.3	1603	47.7
D	7.3±0.4	833	50.1
E	6.3±0.5	633	36.6

*1 mean±SD
*2 swimming ability index; arbitrary unit

Table 3. Behavioral indices and causes of post-stocking mortality or low recapture rate in the released fish of four species studied. Psychological background for each behavior is also listed.

Species	Causes	Behavioral index	Psychological background
Ayu	Downward movement	Spacing behavior*1 Jumping behavior*2	Repulsion
Red sea bream	Predation	Tilting behavior*3	Cautiousness
Flounder	Predation	Feeding behavior*4	Cautiousness
Striped jack	Scattered and lost	Association behavior*5 Diving behavior*6	Composure

*1 Tsukamoto & Uchida[10]
*2 Tsukamoto et al.[8]
*3 Uchida et al.[11]
*4 Furuta[14,15]
*5 Masuda et al.[18]
*6 Masuda et al.[17]

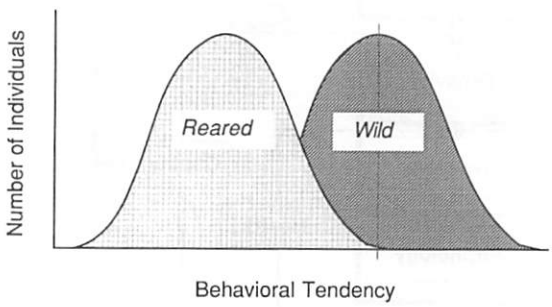

Figure 6. Diagram showing the difference in frequency distribution of some behavioral tendency between wild and artificially reared fish. If assuming that half of the wild fish with lower values in behavioral tendency cannot survive in the field, 100% of reared fish would not be able to adapt to the natural environment and the release of these fish becomes unsuccessful.

tendency is determined not only by genetic factors but also by various environmental conditions. Improving the behavior of the seedlings increases the aptitude of fish for release and decreases the number of fish for release which we should produce in the aquarium, and thus becomes energy saving. Furthermore decreased number of seedlings would minimize the impact of release to the environment.

The process of the study on fish quality is summarized as follows (Figure 7): First of all, we carry out the stocking experiment in the field. We know the process of adaptation or mortality of released fish to estimate the stocking effectiveness. We extract the main cause of post-stocking mortality and choose an appropriate behavioral index which directly links to mortality in the field. Using this index, we quantify the quality of seedlings. In addition, based on such standards of fish quality, we can determine the appropriate releasing tactics such as releasing place, time, season, acclimation procedure and releasing techniques. Another direction of the application of this behavioral index is to determine the factors influencing the

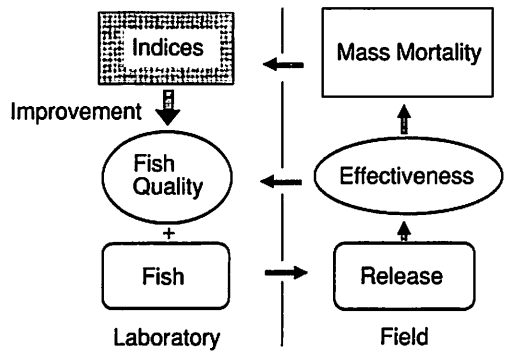

Figure 7. Process and key factors for the study of fish quality.[6)]

development of the behavior, and to improve the fish quality by controlling environmental conditions in a pond. This knowledge will provide us with an understanding to improve the mass production technique. The behavioral approach to fish quality has the potential to present a new horizon in a breakthrough to the research on mass production of seedlings and stock enhancement.

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THE GULF OF MAINE ATLANTIC COD COMPLEX, PATTERNS OF DISTRIBUTION AND MOVEMENT OF THE SHEEPSCOT BAY SUBSTOCK

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ABSTRACT

Atlantic cod (*Gadus morhua*) is a species of groundfish occurring in subarctic to cool-temperate water throughout the North Atlantic. Along the eastern seaboard of the United States, cod occur in discrete stocks. Limited mixing does occur, but the Gulf of Maine stock is the most discrete and enigmatic of the three U.S. stock complexes. Substock structure is, however, poorly understood. One of the locations in the Gulf of Maine where this substock structure is most pronounced is in the Sheepscot Bay. The Bay has consistently been described as an important spawning ground and has periodically been closed to fishing since 1904 to protect spawning aggregations of Atlantic cod. To quantify this substock structure, the Maine Department of Marine Resources tagged 4,191 cod between 1978 and 1983. Over 7.0% of these fish were recaptured up to six years after being tagged. The majority of recaptures were made along the coast and in reasonable proximity to the tagging location inside the 100m isobath. No returns were from south of Cape Cod, Massachusetts, and only three were from Georges Bank and four off the Canadian coast. Long-term tag recoveries, fish recaptured at least one year after release,accounted for 2.7% of the returns and again demonstrated an affinity for the coastal area. Seasonally, returns showed a pattern of concentration indicating prespawning and postspawning aggregations offshore from the tagging site, with returns in the tagging area occurring between May and July. This pattern is not confounded by the distribution of fishing effort and reflects a distinct behavior for this substock of Atlantic cod that includes homing on the spawning grounds. The importance of understanding substock movements is discussed relative to the potential for success of enhancement as a fishery management tool.

INTRODUCTION

Atlantic cod (*Gadus morhua*) range in the Northwest Atlantic from west Greenland to Cape Hatteras.[1,2] Throughout this range they occur in discrete stocks, each having a unique pattern of distribution and movement. In U.S. waters the stocks are identified as (1) Georges Bank, (2) Gulf of Maine, and (3) a southern New England-Middle Atlantic complex. The stock definition is based on tagging studies, parasite infestations, spawning time data, and growth rate analysis.[3] These studies showed substantial mixing between southern New England and Georges Bank cod, but little mixing between those stocks and Atlantic cod in the Gulf of Maine. The Gulf of Maine cod are the most enigmatic of the three stocks. They have been referred to in the fisheries as the "inshore" cod because of their coastal orientation - along the coastal shelf from Massachusetts Bay to the Bay of Fundy, and they comprise several distinct spawning groups that move in the winter and spring onto well-defined grounds in coastal waters to spawn.

Atlantic cod spawning in the Gulf of Maine peaks during December and January in Massachusetts Bay, and in March in Ipswich Bay.[4] These were traditionally considered the largest spawning aggregations in the Gulf. Other productive spawning grounds have been reported off Cape Elizabeth, in Casco Bay, the Sheepscot River, Penobscot Bay, and around Mt. Desert Island, where the spawning season can extend from March to May[1], and in the Bay of Fundy where the season may occur in the fall.[5] What little is known of the spawning and movements of Atlantic cod in the Gulf of Maine comes from a very limited amount of tagging information[5,6], ichthyoplankton studies[4], some anecdotal accounts from the fishery, and the collection of coastal spawning cod for hatchery operations in the early part of this century.[7]

Historically, the only significant tagging of Atlantic cod (about 6000 fish) along the coast of the western Gulf of Maine was done by the U.S. Bureau of Fisheries in the 1930s near Mt. Desert Island. Unfortunately, the results of this tagging have been lost, except for a brief statement, as reported by Wise[6], to the effect that those tagged cod

remained chiefly stationary in that locality and did not mix with cod to the south. In the western Bay of Fundy, in the extreme northeastern Gulf of Maine, over 2000 cod were tagged during several studies (summarized in Hunt and Neilson[5]). Most of these tags were recovered in the western Bay of Fundy and off the western Nova Scotian coast. A tagging program conducted by the Maine Department of Marine Resources in the late 1970s and early 1980s in Sheepscot Bay (Figure 1) has given us the opportunity to examine the movements of one group of Atlantic cod in the Gulf of Maine, which we report on here. Sheepscot Bay is part of a deep, drowned river valley[8,9] in the central portion of the coast. It is fed, in part, by cold upwelled Maine intermediate water from offshore[10] and is a location where Atlantic cod return annually to spawn in May, June, and July. The Sheepscot region has been recognized as a spawning ground for many years and was considered important enough in 1904 to ban "the netting for codfish at the mouth of the Sheepscot River"--the purpose being to "prevent the destruction of female cod, who school in large numbers going up the Sheepscot River to the spawning beds. For some unknown reason this locality seems to be the only known point where a large number of cod are collected at one time for this

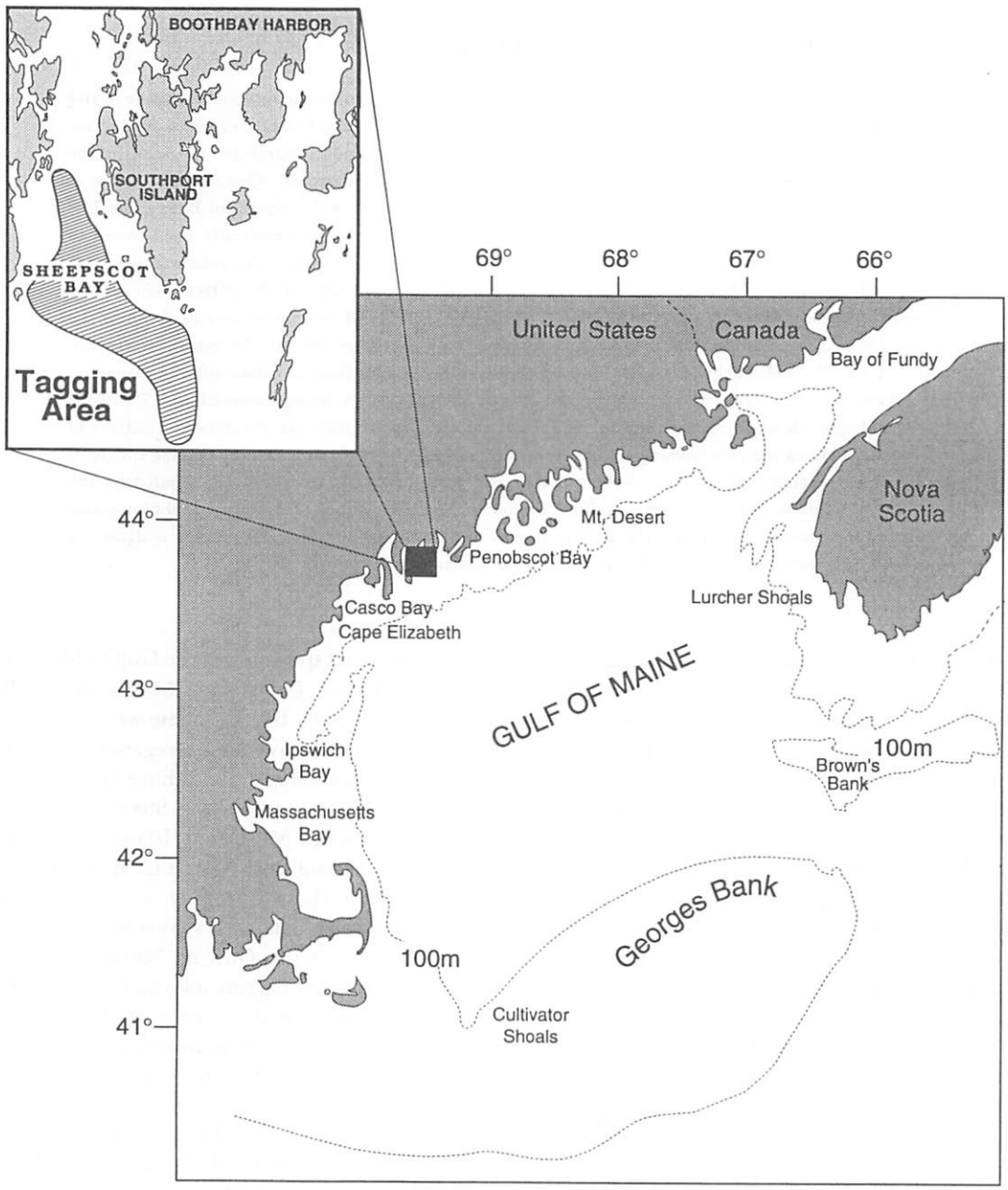

Figure 1. Map of the Gulf of Maine showing the location of Sheepscot Bay, where Atlantic cod were tagged and released between 1978 and 1982.

purpose."[11] Some restriction on cod fishing in the Sheepscot has continued to the present day, as Sheepscot Bay is closed to groundfishing from May 1 to June 30 each year.

The Atlantic cod stock in the Gulf of Maine is presently at historically low levels[12], and the debate over corrective management strategies is intense. Management alternatives include restrictions on overall fishing effort, restrictions on fishing gear, seasonal area closures, possession limits for certain species of fish, as well as the possibility of stock enhancement to hasten stock recovery. The state of Maine has taken the lead in investigating stock enhancement, with the focus on the possibility of re-establishing coastal spawning populations of Atlantic cod by stocking larvae and juveniles in areas that were historically productive spawning grounds and nursery areas. A predictable and accessible spawning location, such as Sheepscot Bay, allowed for tagging a discrete group of spawning cod over an extended period and to examine their dispersal, seasonal movements, and return to spawning locations in subsequent years.

MATERIALS AND METHODS

Tagging operations began in late March 1978, in early May 1979-1982, and in late April 1983, and continued into July of each year. Initially, 262 cod were tagged from a commercial dragger in March 1978 near the Portland light buoy (43° 33′ N, 70° 04′ W). Thereafter all tagging was done in Sheepscot Bay (43° 54′ N, 69° 42′ W), aboard the Maine Department of Marine Resource's *RV EXPLORER* with the exception of a few fish that were tagged in April 1979 between Casco Bay and Boothbay Harbor, Maine (Figure 1).

The fish were caught with small otter trawls, except for a few cod caught by handline. The trawls had 7.6 m headropes and 9.8 m footropes equipped with 15 cm mud rollers. The body of one net, used from 1978 through 1980, was made of 3.8 cm mesh with a cod-end of 3.2 cm mesh. In 1981 through 1983 the net was modified slightly to have a 11.2 cm mesh in the body and 6.2 cm mesh in the cod-end.

Tows were usually of 0.5 to 1.0 h in duration at speeds of 2-2 1/2 knots and in depths of water varying from 22 to 56 m. Fish judged suitable for tagging were immediately placed in a holding tank of circulating seawater. These fish, if behaving normally, were measured, tagged and released. Floy FD-68B yellow T-bar spaghetti tags were imbedded near the base of the first dorsal fin at its posterior edge.

In 1984 a temperature profile was taken at approximately biweekly intervals at a centrally located point in Sheepscot Bay in about 56 m of water. Temperatures were taken from water samples collected with a Niskin bottle from the surface, 20 m and 40 m during January through August, and at 10 m intervals during September and October.

RESULTS

Mature Atlantic cod entered Sheepscot Bay in late April and remained at least until mid-July. Spawning took place at this time as evidenced by the free emission of milt or eggs. A total of 4,191 cod were tagged between 1978 and 1983. Three hundred and four of these were recaptured for a return rate of 7.2%; 255 were from identified catch locations.

The recapture locations of all tagged fish are shown in Figure 2. The vast majority of recoveries were made along the coast from Cape Cod Bay to the Bay of Fundy, and most of these from Cape Elizabeth to Penobscot Bay inside the 100 m isobath. No fish were recovered south of Cape Cod. A few cod were recovered a great distance from the tagging site; three were taken on Georges Bank, one on Browns Bank, and four off Nova Scotia. The most distant recovery was from the Scotian shelf off Halifax, a distance of about 560 km.

Many of the tag recoveries were made during the calendar year in which the fish were tagged and most of them, as would be expected, were recovered close to the tagging site. Several recoveries, however, were made at considerable distances after only a relatively short period from the time the fish were tagged. One recovery was made on Cultivator Shoals, Georges Bank (259 km) after 62 days, one off Lurcher Shoals, Nova Scotia (259 km) after 53 days, and one from Browns Bank (284km) after only 33 days. A few other fish moved fairly rapidly to either the mouth of the Bay of Fundy or Massachusetts Bay.

Long-term recoveries, that is, the fish that were recaptured the calendar year following tagging were of particular interest since this allowed tagged fish to complete a normal seasonal cycle. We recovered 115 tags from fish that had been at large for at least a year, for a return rate of 2.7%. The longest time at large was a fish tagged in May 1979, and landed in Portland, Maine, in March 1985, almost six years later.

The seasonal pattern of long-term recoveries is shown according to recovery location in Figure 3. The Gulf of Maine was divided into areas based on an established system for herring tag recoveries[13]. This divides the coastal zone inside the 100 m isobath into areas that roughly approximate the known locations of cod spawning grounds. Almost all of the long-term recoveries were made along the coast from Massachusetts Bay to Penobscot Bay (areas I-IV) and the area just offshore (VI). The recoveries in area I were made principally during January and February, in area II during January to September, in area

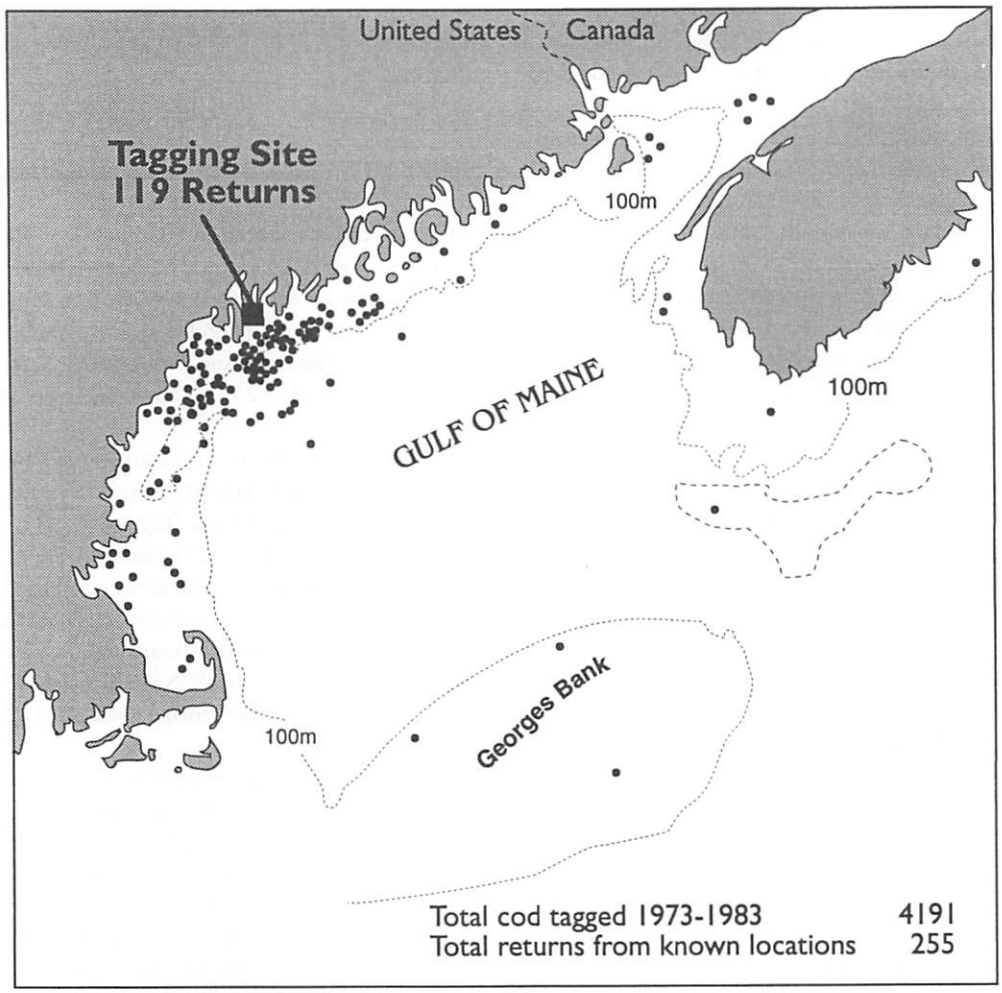

Figure 2. Recapture locations for all tagged Atlantic cod.

III (the tagging site) from May to July, and in area VI primarily in March-April and July-August. The fact that tag returns were highest during April and August in area VI suggests that cod congregate there in prespawning and postspawning aggregations. Almost half (48%) of the long-term recoveries were made during May-July in area III, many of these at the original tagging site. This indicates a high degree of homing to the spawning grounds.

These tag return data are not confounded by the distribution of fishing effort, as reported by the National Marine Fisheries Service for the years 1979 through 1982. The commercial landings of Atlantic cod in the western Gulf of Maine were greatest in the southwest part, from Cape Cod to Jeffreys Ledge (U.S. Statistical Area 514). The cod landings in this region were more than double the catch along the central coast, from Cape Elizabeth to Penobscot Bay, which is the area where tag returns were most numerous (F. Almeida, National Marine Fisheries Service, Woods Hole,Massachusetts, pers. commun.).

DISCUSSION

The Atlantic cod that spawn in the vicinity of Sheepscot Bay constitute a fairly discrete spawning group and are relatively non-migratory, compared to the southern New England Mid-Atlantic cod of Schroeder[14] or some of the northern Atlantic cod stocks off Labrador and Newfoundland.[15,16] During most of the year they are found along the western Gulf of Maine in shoaler, coastal waters or on nearshore banks (areas II, III, and VI in Figure 2) and appear to be distinct from the Bay of Fundy substock of cod recently investigated by Hunt and Neilson.[5] During May into July the Sheepscot cod congregate on the spawning ground, returning to the same location in subsequent years. This supports the conclusion of Wise[6] that Atlantic cod in the Gulf of Maine are comprised of distinct, coastal spawning groups and further suggests that Atlantic cod in the Gulf of Maine should be considered a stock complex rather than a unit stock. Our data show, however, that there is some exchange with

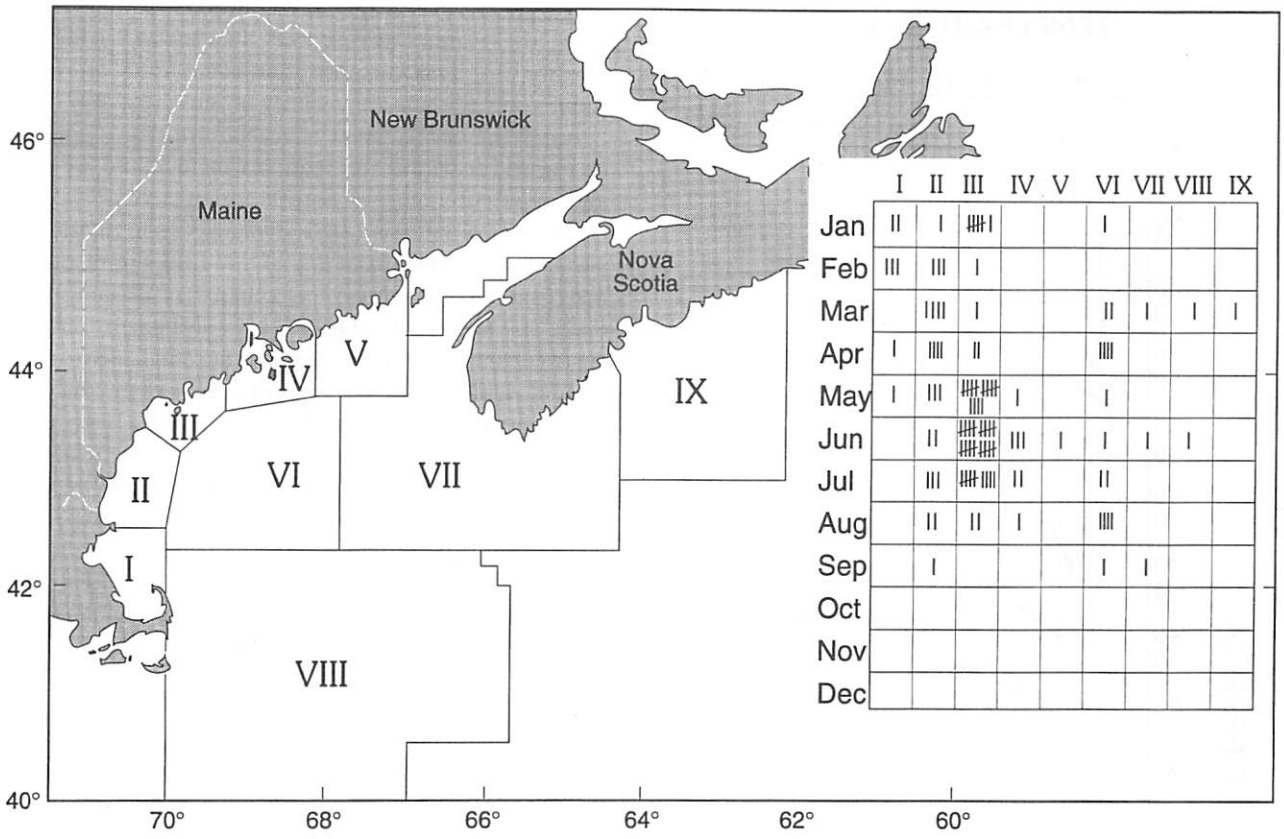

Figure 3. Seasonal pattern of tag returns for Atlantic cod that were captured one year or more after tagging.

other spawning groups, as evidenced by a few recoveries from the Bay of Fundy, Georges Bank, and Nova Scotia.

The results from the tagging work pose several questions about the reproductive strategy of the Sheepscot Bay substock of cod. One of these is why Sheepscot Bay is consistently attractive to spawning cod from May into July? The answer is probably temperature related. Atlantic cod prefer cold water. Their average optimum temperature is around 0-6℃ and they tend to avoid temperatures above 10℃.[17] In addition, the optimum incubation temperature for cod eggs is 5-8℃[1,18] and egg mortality increases significantly at about 10-12℃.[18,19,20] Therefore, any area in the western Gulf of Maine suitable for successful reproduction of Atlantic cod in the spring and early summer is going to have unusually cold water; Sheepscot Bay satisfies this temperature requirement. The deep, drowned river valley that runs offshore from the mouth of the estuary is the source of very cold, upwelled water.[8,9,10] The seasonal temperature profiles in Sheepscot Bay (Figure 4) show that waters deeper than 20 m remain within the optimum range for cod egg incubation (5-8℃) from early May until late July. This coincides with the time that Atlantic cod are spawning there, suggesting that the fish home in on an optimum spawning temperature. Recent work on the northern Atlantic cod stocks[15,16] are further suggestive of the mechanism underlying the movement of cod into the Sheepscot. Rose[15], for example, showed that the Atlantic cod off Newfoundland move from offshore winter spawning grounds to inshore summer feeding grounds along well established "highways" (his term) of stable and optimal temperatures. These cod consistently chose the temperatures found along very specific routes during their migration. Whereas in Newfoundland, cod move inshore in deep water which is warm relative to the cold surface waters, in the Sheepscot the cod move inshore in deep water that is cold relative to the warm, surface water. We suggest the principle guiding the fishes behavior is the same; Atlantic cod seek routes along regular, thermally optimal pathways to move from one phase of their seasonal cycle to another.

The fate of Atlantic cod eggs and larvae in the Sheepscot Bay and how this particular spawning group maintains itself is a more difficult question. Northern Atlantic cod off the Labrador and Newfoundland coasts also exhibit a degree of homing to specific spawning grounds.[21] However, they have been shown to spawn in areas where surface drift can carry the eggs and larvae long distances (up to 900 km)[22] and, according to Myers et. al.[23], the eggs and larvae are not influenced by retention mechanisms, as proposed for Atlantic herring by Sinclair[24] and

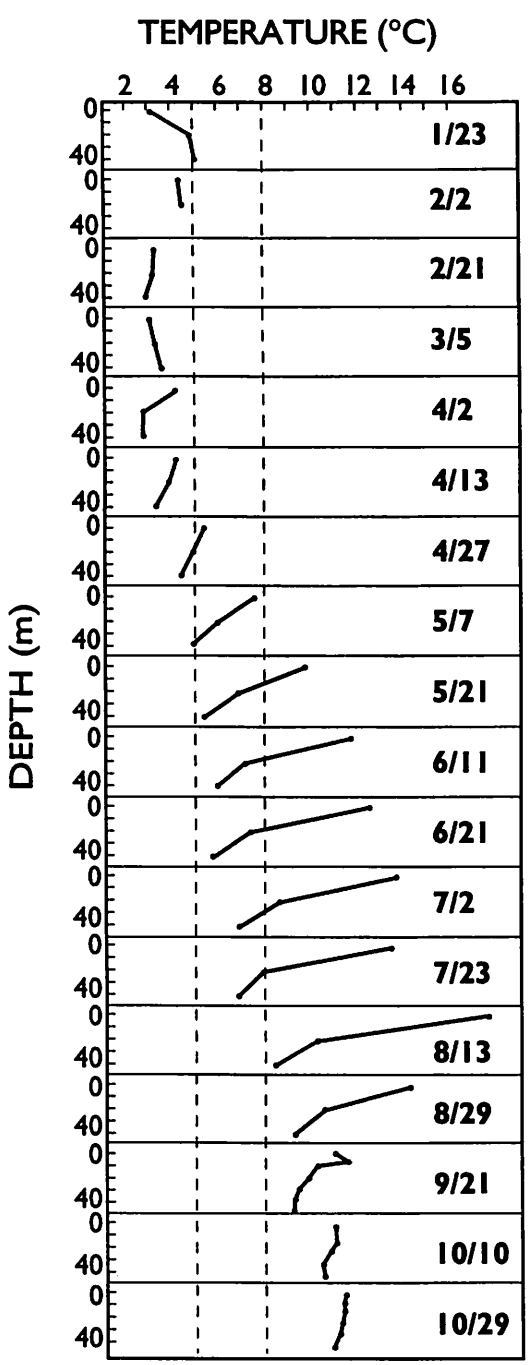

Figure 4. Temperature profile in Sheepscot Bay at approximately biweekly intervals for January through October 1984. Dotted lines indicate the optimal incubation temperature for Atlantic cod eggs.

Sinclair and Tremblay.[25] In the western Gulf of Maine the conventional wisdom has been that pelagic, gadoid eggs and larvae also drift with the prevailing surface currents and reside as early juveniles in the southwest part of the Gulf.[4,26] The problem with this model is that if cod eggs drift away from the Sheepscot spawning grounds, they must survive in the surface waters in June and July when the temperature is well above their lethal limit (Figure 4). This suggests that some type of retention mechanism exists in the vicinity of Sheepscot Bay.

Improved management of coastal fisheries is a necessity on a world scale due to the increasing demand for seafood and apparent limits to natural productivity.[24,27] One component of intensive coastal management is the development of our understanding of the behavior of both fish and fisheries on a finer scale than has previously been done.[9] This is particularly important if one is considering stock enhancement as a management tool to either entirely rebuild depleted stocks or, locally, to reduce the annual variability in recruitment. Enhancement has been successful in many freshwater systems, and for anadromous species, and has more recently been reported to be successful for some estuarine and marine species.[29] Atlantic cod is a marine species which will challenge the skills of the strongest proponents of marine stock enhancement because of the difficulties of rearing significant numbers of larvae or juveniles and because of their complex behavioral and migratory patterns.[15,30] The potential of rebuilding an entire stock complex of Atlantic cod through enhancement is remote to nonexistent and is certainly not currently economical.[31,32] On such a large scale simply reducing fishing pressure would probably be more effective.[33] Nevertheless, the use of cultured animals to rebuild substocks for coastal fisheries has started to be researched and the results show promise (see Leber et al.[34] for a brief summary). For a species such as Atlantic cod, rebuilding specific substocks of fish requires not only knowledge of larval and juvenile culture techniques but also an understanding of the distributional pattern of the fish so that appropriate management measures can be instituted to protect these fish throughout their range. In the case of the Sheepscot Bay cod, for example, there is a spawning closure for the area but it has not been sufficient to protect this substock from suffering the same demise as the entire Gulf of Maine stock complex. The challenge for coastal groundfish stock enhancement programs is to develop a management strategy that will allow an acceptable level of harvest without compromising the reproductive integrity of the substock of fish. Understanding the movement of fish, and ultimately the reasons for the observed pattern, on the scale described herein, is the first step in such a process.

ACKNOWLEDGEMENTS

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OVERVIEW OF AN EXPERIMENTAL STOCK ENHANCEMENT PROGRAM FOR RED DRUM IN SOUTH CAROLINA

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ABSTRACT

Programs to supplement the wild population of red drum, (*Sciaenops ocellatus*), through stocking, are being conducted in several states in the southeastern U. S. In South Carolina, research focused on stocking large (≥100 mm TL) fish which could be externally tagged and reported by anglers. Tagged fish were divided into 3 size categories (≤160, 160-199 and ≥ 200 mm TL) and stocked during different times of the year. During the project (1988-1993), 60,198 fish were stocked. Fishery dependant tag returns demonstrated that spring (March-May) stockings yielded the highest returns with means of 7.2, 3.4 and 2.5% of the fish in the large, medium, and small size categories, respectively, being returned. In contrast, summer (June-August) and winter (December-February) releases resulted in very low returns (≤ 2%) for all size groups stocked. Fishery independent sampling conducted regularly in a stocked estuary, indicated that the contribution of stocked fish to the wild population ranged from 0.3-4.1% during 1989-1994.

A study examined angler reporting of captured tagged red drum. Results indicated that there was no significant difference between the inscriptions "Reward" and "$ 50 Reward" however, tags with "No Reward", were reported significantly less than either reward inscription.

Results of studies evaluating two tag types showed that abdominal anchor tags were superior to T-bar tags for long term (> 1 yr) retention.

An economic model which calculated production and stocking costs for various stocking strategies estimated that the cost per return to the creel ranged from $ 3.52- $ 15.71 for stocking fish in the small size class. Overall, program results indicated that stocking fish could be considered an acceptable fishery management tool.

INTRODUCTION

The red drum, *Sciaenops ocellatus*, is one of the most important marine recreational fish species along the south Atlantic and Gulf coasts of the United States.[1] During the late 1980's, stocks of this species especially in the Gulf of Mexico, were reduced to low levels primarily due to over-fishing.[1,2] As a result, the National Marine Fisheries Service implemented management plans for federal waters along the Gulf and Atlantic coasts, designed to protect against further over-exploitation and to start rebuilding the spawning population.[3] Similarly, the Gulf States Marine Fisheries Commission and the Atlantic States Marine Fisheries Commission instituted regulations to conserve the fish in state waters.

The management plan in South Carolina (SC) has complied with federal recommendations aimed at eventually establishing a 30% escapement rate of juveniles to rebuild the spawning stock biomass. State law has changed a number of times in recent years. Currently, commercial fishing is prohibited and recreational anglers can take 5 fish/day in a slot range (minimum size, 356 mm, maximum, 686 mm TL).

During 1988-1993, the SC Department of Natural Resources initiated a project to examine the biological and logistical issues associated with conducting a stock enhancement program for red drum. This program was based in part on information being collected in Texas and Florida where stock enhancement programs were underway.[4,5] These programs utilized, temperature and photoperiod manipulation to induce spawning of captive red drum,[6,7] as well as intensive pond management for production of juveniles.[8] By 1990, the Texas program was producing 16-30 million, 25-30 mm TL unmarked fingerlings annually, for release in the wild.[4] In contrast, Florida's pilot-scale program was releasing 200-300,000

fish/year in the size range 60-120 mm TL. Each fish was marked with an internal coded wire tag before release.

In SC, the research scale program was focused on releasing larger fish and evaluating this approach as a possible management tool. Specific program objectives included determining: (1) effect of fish size at release on return of stocked fish; (2) optimum season of release; (3) effect of different tag types on retention and fish survival; (4) angler non-reporting level of captured tagged fish; (5) contribution of stocked fish to the local wild population; (6) an estimate of the cost ($) per fish captured. A number of studies were conducted to address each of these objectives.

In contrast to the programs in other states, all red drum released in SC were greater than 100 mm TL and all were externally tagged with individual identifying tags. Fishery dependant (angler participation) and fishery independent data were used to determine capture and contribution levels of the stocked fish. This manuscript provides a summary of the findings of the SC experimental stocking program for red drum.

MATERIALS AND METHODS

General

All fish used in studies were produced from native broodstock which had been conditioned to spawn in captivity using temperature and light control. Fertilized eggs were incubated until hatch in 24℃, 29 g/L saline water and stocked (800,000-1,000,000 fry/ha) in fertilized ponds at the Waddell Mariculture Center in Bluffton, SC. Most fish were spawned in March and April, 4-6 months before the natural spawning season (August-September), to take advantage of favorable outdoor growing conditions in ponds in spring and summer. Fish were harvested after 45-60 days and restocked at lower densities in adjacent ponds for rearing to the size required for particular experiments. Fish were harvested by seining or after pond drainage and mechanically or manually graded into desired size classes. During tagging, fish were maintained in flow-through Colleton River water (25-30 g/L salinity) and anesthetized in a 0.1 g/L solution of Tricaine Methanesulfonate. After tagging, fish were placed in insulated hauling tanks at biomass densities of 60 g/L for transport to their respective stocking site. Water was aerated using compressed oxygen. At the stocking site, fish were acclimated (~15-30 minutes) to ambient conditions before being stocked. Except as noted otherwise, all fish were released in the Charleston Harbor estuary which was approximately 177 km from the production facility (actual tagging and transport time ~4-5 hours).

Two types of tags were used and each tag was inscribed with an individual number and an address for reporting captures. In addition, signs describing the program were placed at all public access points adjacent to the estuary and in local fishing tackle shops. Anglers reporting tags received a hat with the program logo embroidered on it as a reward, and they were also eligible to win a cash reward.

Evaluation of tag types

Two tag types (T-bar and abdominal anchor) were utilized during the program. Both had 45 mm long monofilament streamers (15 mm at base uncovered, 30 mm covered orange message portion) and were manufactured by HallPrint Pty. Ltd. (Australia). The T-bar tag had an 8 mm T-shaped monofilament anchor and was applied with a tagging gun and inserted between the pterigiophores in the dorsal musculature of the fish. This tag could be quickly inserted into the fish. The abdominal anchor tag was inserted in the fish's peritoneal cavity through a hole punched just posterior to the left pectoral fin. The anchor portion was a 17 mm covered T-shaped section of monofilament. This tag required additional time to insert. The two types of tags were tested under actual field conditions and in controlled tank studies.

Field study

Both tags were used during the entire stocking program (1989-1993). A total of 25,738 T-bar and 34,460 anchor tagged fish were released during the program. A subset within the overall program was set up with replicate groups of fish (250-300/tag type/release) with nearly equal numbers of each tag type being stocked between October 1990-September 1992. This was done so that return data from each tag type could be statistically compared. During this period 45 stocking events occurred, encompassing different size classes of fish as well as different seasons of the year. A total of 23,895 fish were stocked of which 11,971 contained T-bar tags and 11,924 had abdominal anchor tags.

Controlled tank studies

The two tag types were tested in two studies conducted in outdoor 3.7×0.9 m deep cylindrical tanks. These tanks received ambient temperature flow-through water (25-30 g/L salinity) from the adjacent Charleston Harbor estuary. During both studies, fish were fed a 38% protein sinking trout ration daily and all fish were individually weighed and measured monthly. The first study was run for four months and examined tag retention and post-tagging survival for smaller fish (mean, 175±SE 1.8 mm TL). These fish were in the small and medium size classes released in the stocking program. Each tag type was applied to three groups of fish (75 fish/tag type/replicate). Three control groups (75 fish/replicate) had an

anal fin clipped for identification. Each tank was stocked with both types of tagged fish and controls (225 fish/tank).

The second study examined long term tag retention and survival among the fish in the largest size class (200-250 mm TL). The study was run for 14 months which is the time period during which the majority of tagged fish released into the wild are reported by anglers. In this study, three replicate groups of 100 fish (mean 249 mm TL ± 2.3 mm), with each tag type, plus 50 fin clipped controls, were stocked in three tanks. Thus, each tank contained fish with both tags and control fish (250 fish/tank). The fish were sampled monthly.

Evaluation of size and season of release

Fish were graded into three size classes prior to tagging and release: small (100-160 mm TL, <40 g); medium (161-199 mm TL, 40-60 g) ; and, large (200-250 mm TL, >60 g). Experimental protocol was that equal numbers of fish in the different size classes would be released during each season of the year. However, small fish were not available for stocking in the summer or fall due to production constraints. Also, only one stocking of large fish was completed in the fall season. Further, number of fish stocked per event varied substantially. For the purpose of this experiment winter=December-February, spring=March-May, summer=June-August and fall=September-November. During handling, water quality parameters (dissolved oxygen, temperature, pH and ammonia) were closely monitored. Post-stocking mortality was evaluated by holding sub-samples of fish from groups to be stocked in the 3.7 m diameter tanks used in the tagging studies for 7 days. Fishery dependant data (angler reports of captures) were correlated with size and season of release to evaluate results.

Determination of non-reporting rate

Information on the reporting level of captured tagged fish by anglers is needed to properly assess return data. A reward study was used to determine the level of non-reporting. One thousand two hundred legal size (≥356 mm TL) fish were separated into four replicate groups (300 fish/group). All fish received abdominal anchor tags inscribed with one of the following messages: No Reward; Reward (message used in marine tagging programs in SC); or $50 Reward (100 fish/message/replicate). The four groups (replicates) were stocked at three sites in Port Royal Sound and one site in Calibogue Sound in Beaufort County, SC. Fish were stocked from boats at each site and dispersed over a wide geographic area (~8 nautical miles/site) in an attempt to limit bias which might occur in the event of multiple captures by a single angler.

Contribution of stocked fish to local wild population

Fishery independent sampling was used to determine the contribution of stocked fish to the wild fishery between 1989 and 1994. Monthly sampling with gill, stop, and trammel nets was part of an ongoing life history project focused on inshore game fish including red drum. Contribution to the wild population was calculated based on the ratio of wild fish to tagged stocked fish present in each sample.

Economic estimates

Estimates of cost/fish returned to the creel were made based on data obtained from size and season of release studies, and combined with cost of production estimates provided by commercial growers. The costs of stocking fish were based on estimates to produce 250,000-2,000,000 juveniles in 2.54 cm size increments with delivery to Charleston Harbor.

Statistical analysis

Data from the tag retention and reward studies were analyzed using analysis of variance. Differences in means were detected with Tukey's test. The tag return data for the abdominal anchor and T-bar tags on fish released in the wild were compared using a T-test. All percentage data were arc-sin transformed prior to analysis. Differences were considered significant at $p < 0.05$.

RESULTS

Evaluation of tags

Field study

Because of unequal sample sizes and other variables, return data from all fish stocked between 1989 and 1993 could not be statistically compared. Returns for the two external tags used indicated that, abdominal anchor tags were returned (1,026/34,460; 2.98%) nearly twice as frequently as T-bar tags (452/25,738; 1.76%). Of the reported captures of T-bar tagged fish, 89.2% occurred within the first year after release, 9.3% returned in year 2, and 1.3% in year 3. In contrast, 77.2% of the abdominal anchor tags reported were returned within the first year with 16.7% occurring during year 2. An additional 6.1% were reported during years 3-5.

The percentage of fish reported captured in the more controlled 2 year field study (October 1990-September 1992) was lower for both types of tags. Returns ranged from 0.0-5.2% with a mean of 1.02% (number returned /release, 3.02 ± SE 0.48) for the T-bar tagged fish and

1.55% (number returned/release, 3.93±SE 0.55) for the abdominal anchor tagged fish. Statistical analysis of the number of returns / replicate release (paired t-tests), indicated that abdominal anchor tags were returned significantly more often than T-bar tags ($p=0.043$).

Controlled tank studies

Analyses of the monthly samples and harvest data for the first four month study indicated that there were no differences in growth among control and tagged fish. Mean retention for abdominal anchor (94%) and T-bar (96%) tags was similar at four months. No differences in mean survival were detected. Control fish exhibited a mean survival of 99% (range, 97-100%) while, tagged fish had a mean survival of 96% (range, 92-100%).

During the initial two months of the second study, mean tag retention for T-bar tagged fish was 84% as compared to 100% for the abdominal anchor tagged fish. During months 3-7, retention remained relatively constant with no further losses of either tag type. However, retention of T-bar tags declined sharply after month seven. At the conclusion of the study (month 14), mean retention of T-bar tags was significantly ($p=0.014$) lower (17.2%, range, 15.4-19.0%) than abdominal anchor tags (100%). Mean survival for the tagged (91.5%, range, 90-94) and control groups (90.0%, range, 89-91%) was similar.

Evaluation of size and season of release

Due to production space limitations, all sizes of fish were not available for every release. The medium size fish were the only category of fish stocked in each season of the year but number of releases varied widely. Because basic statistical assumptions were not met, strict analysis of the size and season data could not be performed. However, the return data for releases of 29,831 (3,650 small, 13,545 medium and 12,636 large) abdominal anchor tagged fish were examined to determine if any trends were apparent.

Season of release appeared to have an effect on return level. In general, highest returns were recorded for fish released in spring. Releases of medium and large fish during this period yielded mean returns of 3.4% (range, 1.1-8.4%) and 7.2% (range, 1.1-17.6%), respectively, while a mean of 2.5% (range, 0.2-8.0%) of small fish were returned (Table 1). Mean returns for all size groups released in the winter were less than 1.6% (Table 1). Summer releases also yielded low mean returns for fish released in the medium (2.0%) and large (2.1%) size classes (Table 1). Subsequent post-stocking mortality studies indicated that most fish died when harvested and released in mid-summer at water temperatures ≥25℃. This finding explains the poor results from stockings conducted in the summer. The one group of large fish released in the fall were returned at a level (9.2%) slightly higher than that recorded for fish released in late spring (Table 1). Medium size fish released during the fall were returned at a level (mean, 3.9%, range, 2.2-5.1%) comparable to similar sized fish released in the spring (Table 1).

Size of fish at release was directly related to return level. Fish from the large size category were returned at higher levels than fish from either the medium or small categories. Maximum returns of 17.6%, 8.4%, and 8.0% for the large, medium, and small size classes, respectively, were recorded for groups of fish harvested, tagged, and released during the spring when water temperature was between 18-24℃. Releasing small fish in the spring provided a greater mean return than releasing medium or large fish in the summer or winter (Table 1).

Determination of non-reporting level

Sixty four percent of the fish returned were caught by anglers who reported catching more than one tagged fish.

Table 1. Mean return rates (%) ±SE for 85 groups (n=29,831) of anchor tagged fish of various sizes (small, 100-160 mm TL; medium, 161-199 mm TL; and, large, 200-250 mm TL) stocked during different seasons of the year. In the table, n=the number of stocking events.

Season	Size Class		
	Small	Medium	Large
Winter	0.5 ± 0.20 n=6	1.0 ± 0.30 n=11	1.6 ± 1.23 n=5
Spring[1]	2.5 ± 0.86 n=8	3.4 ± 0.56 n=17	7.2 ± 1.48 n=13
Summer	..	2.0 ± 0.64 n=7	2.1 ± 0.44 n=12
Fall	..	3.9 ± 0.50 n=5	9.2 ± .. n=1

[1] Includes data for fish stocked through June 10.

Of the 99 participating anglers, 9% reported 31% of the 182 fish captured. Analysis of variance was performed on the pooled return data by tag message and site. No difference in mean reporting level was detected among the "Reward" (19.3%, range, 11-41%) and "$50 Reward" (23.3%, range, 15-42%) messages, but both were significantly greater than the "No Reward" (11.7%, range, 4-22%) message (Table 2).

Returns from fish stocked at three sites (range, 10.0-15.0%) were not statistically different. However, significantly ($p=0.0013$, $n=3$) more returns (35%) came from the site which was near the town of Port Royal and which had higher fishing pressure.

When data for fish reported singularly by anglers were grouped and analyzed, results were similar to that of fish reported from multiple captures.

Contribution of stocked fish to local wild population

Randomized fishery independent samples in the Charleston Harbor estuary between 1989 and 1994 verified that stocked fish contribute to and occur with wild stocks. Annual percentages of stocked fish in samples ranged from 0.3-4.1% (mean, 1.4%) (Table 3). The peak number of captures of stocked fish occurred in 1992 when 94 tagged stocked fish were captured during sampling (Table 3).

Economic estimates

Two commercial hatcheries responded to questionnaires requesting delivered prices for quantities of juvenile red drum ranging from 250,000-2,000,000 in 2.54 cm increments between 5.1-12.7 cm. One respondent was a hatchery in Texas and the other in South Carolina. Due to the longer distance involved, the prices quoted for the Texas supplier were higher. Price per individual ranged from $0.14 for 2,000,000 5.1 cm fish from a South Carolina hatchery to $0.45 for 250,000 12.7 cm fish shipped from Texas. There was approximately a 14% discount for purchasing the largest quantity.

Estimated stocking cost data (for fish in 10.2 and 12.7 cm TL size classes) were combined with return data based on these size fish (smallest size group released) and season of release. Based on abdominal anchor tagged fish, the highest mean (2.5%, range, 0.1-8.0%) returns for the small size class occurred in late spring. Using the quantity discount cost and the highest return level (8%) recorded for this size class, cost/return was $3.52 for 10.2 cm TL fish and $4.43 for 12.7 cm TL fish. However, if the mean (2.5%) return level was used, cost/return increased to $12.90 and $15.71 for the 10.2 and 12.7 cm TL fish, respectively.

DISCUSSION

Interpretation of results from this research study are conservative as the return levels no doubt did not include all captured stocked fish (e.g. tags shed, not observed, not reported). Fishery independent data clearly indicated that the stocked red drum grew and behaved similarly to wild fish and provided a contribution of up to 4.1% to the wild population. Weaning from a strictly pelleted ration to

Table 2. Mean return rates (%) for red drum ($n=1,200$, 401 cm TL) released in replicate groups at three sites in Port Royal Sound and one site in Calibogue Sound with tags inscribed with one of three reward messages.

Reward Message	Mean[1]	Range	±SE
$50 Reward	23.3 A	15.0 - 42.0	6.4
Reward	19.3 A	11.0 - 41.0	7.3
No Reward	11.8 B	4.0 - 22.0	3.8

[1] Means followed by the same letter are not significantly different ($p \leq 0.05$).

Table 3. Contribution of stocked red drum to the wild population in Charleston Harbor estuary.

Year	released (#)	sampled (#)	Contribution	
			(#)	(%)
1989	4,145	897	7	0.8
1990	5,961	784	30	3.8
1991	11,279	1,209	4	0.3
1992	15,409	2,265	94	4.1
1993	14,957	2,496	33	1.3
1994	0	2,246	22	1.0

Table 4. Estimated cost ($) per returned fish based on the mean (2.5%) and maximum (8.0%) returns recorded for similar size fish stocked in the spring and estimated costs provided by a SC commercial hatchery. Prices for larger size fish were not quoted.

Quantity (#)	Total Length (cm)	Cost ($) /return	
		2.5%	8.0%
250,000	10.2	12.90	4.03
	12.7	16.18	5.06
2,000,000	10.2	12.90	3.52
	12.7	15.71	4.43

natural foods occurred rapidly as some fish were captured within 1-2 days of release by anglers using natural baits and artificial lures.

Selection of proper tags to externally mark fish for long term identification is extremely important. Results of our studies showed the value of conducting long term studies (> 1 year) and indicated that the abdominal anchor tag provided much better long term retention than the T-bar tag. This was supported by field testing which showed that almost all fish, reported after 3-5 years at large, contained abdominal anchor tags. Neither tag type affected growth or survival under controlled tank conditions.

Since much of the data collected was from fishery dependent sources it was necessary to evaluate level of angler non-reporting of captured tagged fish. Studies in other states have observed non-reporting rates by marine anglers of > 50%.[9,10] Our angler non-reporting study indicated that, the offer of a reward was very important. This finding has also been reported by Butler[11]. Offering no reward resulted in significantly fewer returns than offering either a non-specific reward or a $ 50 reward. The returns for tags inscribed "Reward" were not significantly different from the offer of "$ 50 Reward". This finding was encouraging as most marine fish tagging programs in SC offer a "Reward" for captured fish. The previously assumed 50% non-reporting level did not appear to be valid based on our results. However, a study with ducks suggests that $ 50 is too low a reward to expect a 100% reporting level.[12] Our study also demonstrated the influence of stocking location on return level. Stocking near an urban area resulted in a 35% return level while stocking in less accessible fishing areas provided lower return levels. The return levels observed for legal size stocked fish released in this experiment are similar to those observed for wild fish which are tagged and released in SC.[13]

Due to production constraints caused by limited pond space and avian predators, studies focused on evaluation of size and season of release could not always follow rigorous experimental protocols. However, the trend was clear that larger fish are returned more frequently than smaller size fish, a finding similar to that reported by Willis et al.[5]. We also found that spring and fall releases provided higher returns than stocking during winter or summer.

Based on reports of economic impacts of fishing for red drum in Texas, Matlock[14] estimated cost per returned fish in SC appeared acceptable. It was not possible to obtain comparable data for stocking fish in the medium and large size classes. From a cost standpoint, production of smaller fish seems more efficient and less risky and is the approach considered most beneficial in Texas.[4,15] As mentioned earlier, bird predation of pond reared fish became an increasing problem,[16] as was concern about low temperature induced kills,[17] during over-wintering of fish in outdoor ponds. Shorter duration of captive rearing used to produce small fish may also have some beneficial genetic considerations. However, a program based on stocking very small fish can not be accurately assessed without a method of identifying the contribution of stocked fish to the wild population.

Efforts have been undertaken to develop the use of natural marks on otoliths and scales for marking small fish. Otolith microstructure has been used as a "natural tag" and to distinguish hatchery reared and wild fish.[18, 19, 20] Recently, we observed that the growth pattern on the otoliths of out-of-season spawned red drum could be used as a mark to identify stocked fish.[21] In fact, this technique was recently used to examine archived otoliths from two year classes of "wild" fish collected during routine fishery independent sampling. This examination revealed that 4% of red drum formerly classified as "wild", were actually stocked fish that had lost their tags prior to capture. Recently, in SC, a stocking experiment has been initiated where 200,000 to 500,000 small juveniles (25-40 mm TL) are being marked with oxytetracycline HCL,[22] and stocked in the fall and spring to evaluate seasonal effects and to validate utility of using the otolith marks for long term identification.

In summary, stocking red drum was shown to be a valuable management tool for use by fishery managers. However, issues relative to the interaction of stocked fish on wild populations (e.g. supplementation, competition, replacement) and ecosystem carrying capacity still need to be addressed.

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MARINE STOCK-ENHANCEMENT POTENTIAL WITH STRIPED MULLET, *MUGIL CEPHALUS*, IN HAWAII

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ABSTRACT

Three common methods have been used to replenish depleted stocks: regulating fishing effort; restoring degraded nursery and spawning habitats; and increasing recruitment through propagation and release. Declines in world fishery landings have prompted new interest in using cultured fishes to help replenishdepleted stocks. With advances in tagging methods and aquaculture technology for marine finfish, stock enhancement through the release of hatchery-produced juveniles is becoming one of the solutions for replenishing depleted coastal fishes. The hypothesis that hatchery releases can increase population size has at least two corollaries that need to be tested: (1) released cultured fish survive, grow and contribute to natural recruitment, and (2) cultured fish do not displace wild stocks.

The concept that depleted populations of marine fishes can be revitalized using cultured fish is being tested in Hawaii, where landings of coastal species have declined by 80% since the turn of the century.[1] Striped mullet (*Mugil cephalus*) and Pacific threadfin (*Polydactylus sexfilis*) have been selected as top priority species for stock enhancement research. Intensive studies on striped mullet stock enhancement have been carried out at The Oceanic Institute in cooperation with the State of Hawaii. The research approach in Hawaii followed three steps: select test species, evaluate release strategies with pilot releases, and conduct a test release using optimal release protocol. During pilot releases, three important factors (fish size-at-release, release habitat and release season) were evaluated.

To evaluate the initial success of hatchery releases in Hawaii, we tracked survival of released fish prior to and after their entry into the fishery. Each year, beginning about 2 weeks after releases, monthly cast-net collections were made in six nursery habitats over about a 10-month period to monitor recapture rates, growth and dispersal of the juvenile cultured fish.

Recapture rate of cultured fish during the juvenile nursery stage of the life cycle was directly affected by the release site, fish size-at-release and the seasonal timing of releases. Over 30,000 juveniles stocked in 1990 (but not in a nursery habitat preferred by striped mullet) apparently suffered complete mortality. However, there was good survival of fish when they were released into documented nursery habitats of wild mullet. Greatest recovery of the smallest fish released (individuals $<$60 mm) occurred following spring releases, which coincided with peak recruitment of similar-size wild *M. cephalus* juveniles. After summer releases, recapture rates were strongly affected by fish size-at-release, with a critical release size of 60 mm total length (the smallest size released that was subsequently detected in the fishery). We hypothesized that survival of released cultured fish will be greater when releases are timed so that fish size-at-release coincides with modes in the size structure of wild stocks.[2]

Following pilot hatchery releases from 1990 to 1993, striped mullet fisheries in Kaneohe Bay, Hawaii, were also sampled to recover cultured fish from the bay-wide catch. Direct sampling of 181 fishing trips resulted in recovery of 211 cultured striped mullet. By autumn 1994, cultured fish comprised 13.0% ($\pm$2.8%) of the commercial mullet catch in Kaneohe Bay, and the percentage was increasing logarithmically.

To optimize effectiveness of stock enhancement as a fishery-management tool, pilot release-recapture experiments should be conducted to evaluate effects of release protocol on recovery of released animals. For example, In Hawaii, hatchery production cost-per-fish-caught in the fishery was lower for releasing 70-85mm size fingerlings compared to 45-60mm fingerlings when releases were conducted in summer. By refining release protocol over a 3-year period, proportions of cultured fish in nursery habitats 10 months after release increased from 3% to 10% and finally to 50% of the total striped mullet (wild and cultured) collected in net samples.

At least three measures were needed to describe hatchery effect: (1) hatchery contribution (% cultured fish in samples), (2) catch-per-unit-effort for cultured and wild striped mullet, and (3) recovery rate (no. captured/no. released). Our studies demonstrate how survival of cultured fish in coastal

nurseries can be significantly improved using information from pilot release experiments to revise release parameters. Results at The Oceanic Institute have shown that the targeted inshore fish population can be increased through the release of hatchery-produced juveniles. However, to provide adequate conservation of the wild stocks we are attempting to replenish, a responsible approach should be used for any stock enhancement activities.[3]

INTRODUCTION

Decades after initial efforts failed to show any effects (see Richards & Edwards[4]), attention is being refocused on the concept of releasing cultured organisms to supplement and restore declining coastal fisheries (marine stock enhancement). Fishery biologists now have access to the technology needed to evaluate stock enhancement potential. As coastal fisheries face severe over-exploitation and depletions worldwide,[5] it is time to resolve lingering questions about whether marine stock enhancement can help recover lost recruitment potential and supplement abundances of wild stocks.

There is little published quantitative evidence that stocking cultured marine organisms (that *spawn* in seawater) into coastal areas results in recruitment of released individuals to fisheries. Notable exceptions include ongoing work in Norway and Denmark with cod, *Gadus morhua* L. (e.g. Svåsand et al.[6]; Nordeide et al.[7]; Danielssen & Gjøsæter[8] ; Støttrup et al.[9]), in Japan with the flounder "hirame"-- *Paralichthys olivaceus* (e.g. Kitada et al.[10]) and the scallop *Patinopecten yessoensis* (e.g. Honma[11]), in the U.K. and France with lobster, *Homarus gammarus* L (e.g. Bannister & Howard[12]; Latrouite & Lorec[13]), and in Spain with turbot, *Scophthalmus maximus* L. (Iglesias & Rodríguez-Ojea[14]). Very few studies have quantified the percent contribution of cultured marine finfish to commercial fishery landings (but see Svåsand et al.[6]; Kitada et al.[10]). Although, published accounts of effects of marine hatchery-releases on fishery landings are uncommon, considerable work to quantify marine, stock-enhancement recruitment dynamics has begun within the past decade (e. g. Tsukamoto et al.[15]; Svåsand & Kristiansen[16]; Barlow & Gregg[17]; Jørstad et al.[18]; Stoner[19]; and other studies in symposia proceedings edited by Lockwood[20], Danielssen et al.[21], and Schramm & Piper[22]).

The marine stock enhancement concept is being evaluated in coastal waters in Hawaii, USA. Since 1989, a research program, titled "Stock Enhancement of Marine Fish in the State of Hawaii (SEMFISH)," has been conducting test releases in Hawaiian estuaries and shoreline marine habitats.[2, 23-25]

In 1988, a semi-quantitative, species-selection process was used to identify marine finfish for stock enhancement research in Hawaii. The selection process involved federal, state, non-profit and local representatives of the Hawaiian fisheries community. The marine fishes, Pacific threadfin (*Polydactylus sexfilis* C., V.) and striped mullet (*Mugil cephalus* L.) were the two highest ranked species.[26] After species were prioritized, and mass-culture techniques became available for striped mullet,[27] pilot release-recapture experiments began, using striped mullet as the initial test species for SEMFISH research. In 1993, following development of production techniques for Pacific threadfin,[28, 29] the first test releases began with that species.

The hypothesis that hatchery releases can help increase marine fish populations has at least two corollaries that need to be tested. One is that cultured fishes released into coastal waters actually survive, grow and contribute to juvenile recruitment and fishery landings. The other corollary is that cultured fish do indeed increase abundance rather than displace wild stocks. These two postulates are basic assumptions of stock-enhancement theory, yet both remain largely untested in coastal ecosystems (i.e. with organisms that reproduce in marine environments). The former corollary is the focus of this paper, the latter is considered elsewhere.[24]

To design a rigorous test of the marine stock enhancement concept, data would be needed from pilot releases to define effective release strategies. Fish size-at-release (SAR) and the timing of releases were important choices that needed to be made, as Hager and Noble[30] and Bilton et al.[31] had already shown with coho salmon released into streams in the Pacific northwest. Releasing fish into coastal environments would also require careful consideration of release habitat. If any of these three variables affected survival of released fish, then they would also affect the power of any test of stock-enhancement potential. In Hawaii, a series of pilot release experiments were conducted to identify effects of release magnitude, SAR, release habitat and release season on survival and contribution of cultured striped mullet to wild stock abundance.[2, 23] The results of those pilot releases were used to design the test of the first corollary -- that cultured fish make a substantial contribution to a marine fish population in Hawaii.

As pilot release-recapture experiments began in Hawaii, Tsukamoto et al.[15] published results that indicated SAR affected survival of red sea bream, *Pagrus major*, juveniles released into News Bay, Japan. In 1990, Svasand and Kristiansen[16] showed similar results with cod, *Gadus morhua*, released into Norwegian fjords. In 1990, a similar pattern was observed in Hawaii following summer releases of around 40,000 tagged striped mullet into each of two

embayments on Oahu, Maunalua Bay and Kaneohe Bay.[23] Work with striped mullet revealed that recapture rates approached zero when cultured fish smaller than 60 mm total length (TL) were released in summer or fall months. These results ruled out the alternative of stocking newly hatched fry or postlarvae in an experimental test of the stock-enhancement concept in Hawaii. Pilot releases in Hawaii also revealed that survival of cultured mullet was strongly affected by release habitat and release season.[2, 23, 32] Hatchery-release studies with marine fishes in Norway, Florida and California,[16, 33, 34] and of cultured conch released in the Caribbean[19] have shown substantial affects of release strategies on survival in coastal environments.

The Hawaiian studies of recruitment success of released cultured juveniles were conducted in and around fresh water tributaries, the preferred nursery habitat for striped mullet. Striped mullet are catadromous and begin to move out of their nursery habitats as they approach maturity.[35] In Hawaii, yearling juveniles begin to move out of the intertidal zone and out of shallow shore zones in streams by around February or March.[23, 36] Striped mullet reach advanced sexual development in fresh water, but must migrate to the sea to spawn.[35] Annual recruitment into inshore nursery habitats of young-of-the-year wild mullet occurs in spring in Hawaii.[36]

In Hawaii, striped mullet are fished by commercial and subsistence net fishers as well as by recreational pole-and-line fishers. However, recreational fishing for this species has long been on the decline throughout the islands. Historically, creel surveys in Hawaii began as a compilation of creel data collected ad hoc from coastal areas, and were often conducted without stratified statistical consideration and only for brief time periods. The need to develop a more quantitative inshore creel survey was identified as a priority by the Hawaii Division of Aquatic Resources (HDAR) in 1990.[37]

This paper describes results from the studies designed to recover data on survival of released hatchery fish in their nursery habitats, and from a study to document the impact of hatchery releases on the commercial mullet fishery in Kaneohe Bay. Our studies revealed that pilot-scale hatchery releases conducted from 1990-1993 contributed significantly to recruitment in juvenile nursery habitats and, eventually, to yields in the mullet fishery.

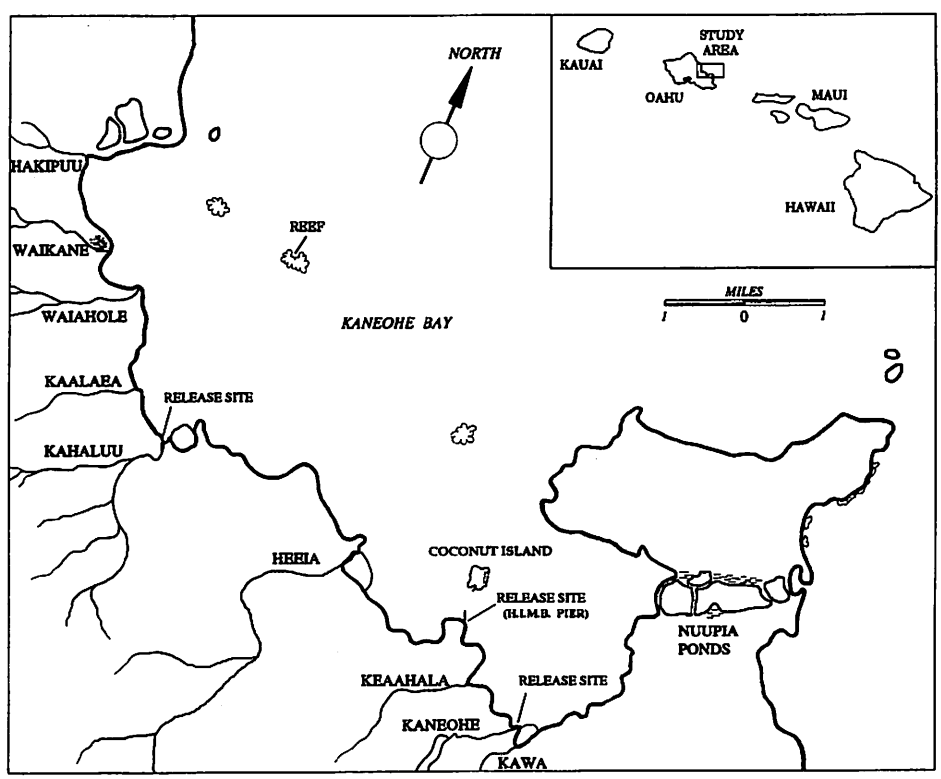

Figure 1. Map of the study area in Kaneohe Bay. Releases were conducted near the mouths of Kahaluu Stream and Kaneohe Stream, and near the Hawaii Institute of Marine Biology Pier (HIMB). Recapture collections were conducted in streams throughout the Bay and on reef flats in the vicinity of stream mouths.[25]

MATERIALS AND METHODS

Between June 1990 and August 1993, approximately 243,500 cultured striped mullet were marked with binary-coded wire tags,[38, 39] and released into Kaneohe Bay (Figure 1; see Leber[23]; Leber et al.[2, 24, 25] for details). The cultured fish were produced from wild parental stock at The Oceanic Institute in Waimanalo, Hawaii (USA), and reared to 45-130 mm long fingerlings, which were graded into five size groups prior to release. The coded-wire tags (CWT) identified fish size-at-release (SAR), release site, release magnitude, and release lot (date).

In 1990 and 1991, release-recapture experiments were conducted to develop release protocols for testing the marine stock-enhancement concept with striped mullet in Kaneohe Bay, Hawaii.[2, 23] This site is the largest estuary in Hawaii, and extends 14 km along the island of Oahu's windward (east) coast. The resulting release-recapture data on fish size-at-release, release habitat, and release-season effects on survival were used to redesign release strategies. Test releases were then conducted in 1992 and 1993 to evaluate key assumptions about the impact of hatchery releases on juvenile recruitment in Kaneohe Bay.[24, 25]

In 1990, the effects of release habitat and fish size-at-release on survival of cultured fish were evauated in Maunalua Bay (south shore of Oahu) and in Kaneohe Bay (eastern, windward coast of Oahu). About 85,000 fish, ranging in size from 45 to 130 mm TL (total length) were harvested from culture tanks and transferred to 40,000-liter holding tanks. These fish were graded into five size groups, tagged, then released during the summer into both bays.[23]

In 1991, a factorial-design release-recapture experiment was performed to compare interactive effects of release season and fish size-at-release upon growth and survival of about 90,000 cultured striped mullet in the wild. Striped mullet, ranging in size from 45 to 130 mm TL, were harvested, graded into five size groups, tagged, and then released into Kaneohe Bay; half were released in May, the other half in July.[2]

In 1992, release strategies were redesigned based on survival data for juveniles following the 1990 and 1991 test releases. Using the revised release protocol, about 80,000 juveniles were tagged and released to evaluate the potential to use hatchery releases to significantly increase striped mullet recruitment in Kaneohe Bay. Criteria for success were (1) cultured fish released in this study comprise a substantial proportion (at least 20%) of the juvenile striped mullet in net samples 4 months after release, (2) persistence of cultured fish in net samples throughout the study, and (3) growth comparable to measured rates in wild juveniles. If these criteria were met, it would be reasonable to assume that cultured fish had substantially affected juvenile recruitment at the study site. In 1993, an experiment was conducted to evaluate if the hatchery releases were actually adding to fish production, rather than displacing wild striped mullet (see Leber et al.[24]).

To evaluate the full potential of marine stock enhancement, results of pilot releases need to be quantified at various stages of the life cycle. Following the pilot studies above that evaluated recruitment success and survival patterns during the juvenile stage, surveys of fishermen were initiated in mid 1992 to examine growth and survival after cultured fish dispersed from their nursery habitats into habitats occupied by adults.[32] The primary objectives were:

1. to determine if cultured striped mullet released as juveniles could survive and grow to mature adults and recruit to the local spawning stock;
2. to identify the percent contribution of cultured fish in the local fishery; and
3. to determine if survival patterns of released fish changed after the juvenile stage, by comparing survival patterns of adult cultured fish captured in the bay-wide fishery to results from previous studies of juveniles in Kaneohe Bay nursery habitats.

Monitoring

Sampling juvenile cultured fish in nursery habitats

Beginning in 1990, we monitored abundances of hatchery-released and wild *Mugil cephalus* in Kaneohe Bay monthly for 10 to 11 months after releases each year by sampling with cast nets. Recaptured tagged fish were removed from collections and returned to the laboratory for tag analysis. The first field collection after spring or summer releases usually began two weeks after the middle release lot (lot 2, out of 3 lots per season) was planted.

Each monthly collection was conducted over approximately a two-week period. Collections were made at six nursery sites (sampling stations) within Kaneohe Bay. Collections were done during the day over about an 8-hour period at each sampling station. Stations were established in the vicinity of documented striped mullet nursery habitats at various tributaries located throughout the bay; [23] six streams in Figure 1: Waiahole, Kaalaea, Kahaluu, Heeia, Keaahala and Kaneohe stream.

To standardize collection effort, at each station two substations were sampled --one substation was established upstream, the other near the mouth of the tributary. Within substations, 15 cast net throws were made. To broaden the range of microhabitats and fish size-ranges sampled, two sizes of cast nets were employed. Ten of the 15 casts per substation were made with a 5-m diameter, 10-mm mesh net, and five casts were made with a 3-m diameter, 6-mm mesh net. Thus, a total of 180 casts were made each month (120 casts per month in 1992, when the number of nursery habitats sampled was reduced from six to four).[2, 23, 25]

Placement of net samples was stratified over observed

schools of striped mullet juveniles. Completely random sampling in preliminary collections yielded few wild striped mullet and very few tagged individuals. Striped mullet schooled in fairly low densities within these clear-water nursery habitats, and our stratified-random collections targeted those schools. Nevertheless, the sample data used to determine proportions of tagged versus untagged mullet were randomly distributed, because we had no a-priori indication that schools, once sighted, contained tagged individuals.

Sampling cultured fish in the fishery

A sampling program was designed to recover cultured striped mullet from the fishery in Kaneohe Bay and document the hatchery contribution to catch per unit effort (CPUE) in the fishery during 1992, 1993 and 1994. Sampling initially targeted recreational and subsistence fishers, but was redesigned in 1993 to target greater catches in the commercial fishery.

Creel survey activities followed a structured sampling schedule to obtain basic catch information. Initial surveys were stratified around area, time, and gear to provide catch and effort data. The sampling design also evaluated participation levels (total effort by location and time of day). Data collectors obtained fishing trip information, catch data, and tagged mullet from both completed and incomplete fishing trips. Striped mullet catches were sampled for tagged fish using a tag detector. Estimates of catch per unit effort (CPUE) were obtained by dividing measured harvest by measured effort.

A public awareness program was initiated to highlight program objectives and to elicit support from the fishing community. Fishers were contacted and informed of stock enhancement activities. Additional insight was sought from fishers regarding current and historical fishing trends within Kaneohe Bay. A list of potentially cooperative fishers was identified and compiled, and they were contacted to enlist their assistance in obtaining additional catch and fishing effort data.

Modified creel sampling approach targeting the most successful fishers

After creel census activities in summer and autumn 1992 resulted in catch data totalling only four striped mullet, the approach was modified to include sampling of commercial fisheries. The survey was confined to Kaneohe Bay. Project biologists contacted local fish wholesalers and retailers at the Honolulu fish auction at the beginning of the fishing season in March 1993. Through informal interviews conducted at the auction, better insight was gained about the structure of the fishery. Most striped mullet fishing on Oahu was commercial, and many "subsistence" fishers held commercial licenses to sell their catch.

A new approach was developed, aiming to identify the principal commercial fishers for striped mullet in Kaneohe Bay, and to obtain the largest sample possible from that fishery. Effort was refocused in two directions:

1. sampling the Kaneohe Bay striped mullet catch sold in local fish markets;
2. direct sampling of the commercial catch in Kaneohe Bay.

Discussions with local fish-market owners were expanded to gain their cooperation and sample catches of local striped mullet brought to their markets. Fish-market owners were contacted weekly to sample mullet catches. Maintaining regular contact with fish-market owners also expanded the list of identified commercial striped mullet fishers in the bay, as a trusting relationship was developed with the owners.

Next, direct contacts were made with commercial fishers in Kaneohe Bay to inform them of SEMFISH activities and to elicit their cooperation in retrieving tagged adult striped mullet. At this point, the fish-market owners were invaluable in their help with obtaining access to commercial fishers. After developing fisher contacts in the field, a SEMFISH researcher either participated as crew in fishing excursions and sampled the mullet catch *in situ*, or waited at the dock and sampled the catch. This generated data on gear type, time spent fishing (effort), total catch per trip, and proportions of cultured and wild fish caught. These data provided reliable estimates of CPUE (for fishers who allowed SEMFISH biologists to participate as crew members) and provided percent contribution of cultured fish in the catch.

The modified creel surveys were run concurrently during the open fishing season for striped mullet, from March through November, in 1993 and in 1994. Cultured fish were detected in catch samples from surround nets, gill nets and cast nets using field sampling detectors (Northwest Marine Technology Inc., Shaw Island, WA, USA) to detect coded-wire tags. All tagged fish were provided without cost by fishers when project staff worked as crew on board vessels; otherwise, wholesale price was paid for tagged fish.

Some fishers were unwilling to cooperate directly, but their catches were often sampled indirectly at the fish-markets they supplied. Information was often provided by cooperative fishers when a commercial catch by uncooperative fishers was taken to market. On many such occasions, it was possible to track these catches to the receiving fish-market and sample them there.

To increase project awareness, project biologists conducted informal meetings with the Kaneohe Bay fishing community, and provided an informative pamphlet soliciting help from fishers in retrieving tagged adult fish. Pamphlets were also widely distributed to local fishing-tackle shops.

Tag-data retrieval

All striped mullet sampled were measured and checked

for tag presence using a field sampling detector (Northwest Marine Technology, Inc., Shaw Island, WA, USA). Tagged mullet were placed on ice and returned to the laboratory where the tags were recovered and each fish was weighed and measured.

Treatment identifications were made based on the tags retrieved from recaptured fish. In the laboratory, tags were located and extracted from the snout using a field sampling detector and a binary search pattern. Tags were decoded using a binocular microscope (at 40x). To verify tag codes, each tag was read twice (once each by two different research assistants).

Data were analyzed using Systat.[40] A randomized-block factorial analysis of variance (ANOVA) was used to compare means. Systat Basic was used to write tag decoding algorithms. For each recaptured fish, the algorithms identified batch size, release date (lot), release site, size-at-release, and release season, based on the tag codes. Average growth rate was computed for each individual recaptured by dividing change in length (the difference between length at capture and the median length within SAR group) by weeks at sea. An error-check algorithm was also written to help identify if errors were made in reading tag codes. Proportions were arc-sine transformed prior to statistical analysis. Variance estimates are expressed throughout as standard errors (with the number of experimental replicates, n, =number of release lots).

RESULTS

Pilot release to develop effective release strategies.[2]

Recapture Summary

Following the pilot release experiment in 1990, which showed that recapture rate of cultured fish was directly related to fish size-at-release,[23] we intensified our experimental evaluation of size-at-release effects in 1991. The results are explained in detail here. Cultured fish were easily recovered from striped mullet nursery habitats. For example, after releases in 1991, 2,511 cultured striped mullet were recaptured in monthly cast-net samples. Based on a 98.6% average tag retention rate, number of cultured fish recaptured in our cast net samples can be extrapolated to 2,546, or 2.8% of the fish released in 1991. Total number of tagged fish in samples decreased over the 11-month monitoring, but were fairly constant during the last 7 months of the study (when numbers of tagged fish ranged from 49 to 117 individuals).

Tagged fish comprised between 8% and 48% of the striped mullet captured in monthly samples (from all stations combined). Percentage of cultured fish in samples was greatest at Kaneohe Stream, where contribution rates declined from 76% following the May release to 41% by the end of the study. Although numbers of tagged fish collected at Kahaluu Stream were often similar to those for Kaneohe Stream, there were always greater numbers of wild fish in collections at Kahaluu Stream.

Impact of Release Season

Recapture Rates and Contribution Rates. When size-at-release was not considered, the contribution of cultured fish to recruitment appeared to be unaffected by release season. Release season had no significant effect on mean recapture rates over time (ANOVA, $P>0.54$, data from all size-at-release intervals combined). After 3 months in the wild, mean numbers of cultured fish in samples varied between about 10 and 27 individuals per release lot throughout 36 weeks (Figure 2). However, as shown below, recapture rate was in fact dependent upon the interactive effect of release season and size-at-release. (Note: for evaluating release-season effect, data can be compared only through 35-36 weeks following releases, the length of time fish were monitored after summer releases; by the end of the study, fish released in the spring had been in the wild for an average of 45 weeks, 10 weeks longer than those released in summer).

Dispersal Patterns. There were no clear seasonal trends in dispersal patterns. Cultured striped mullet showed a strong tendency to remain in the vicinity of release sites, regardless of release season or size-at-release. Few of the 2,511 tagged fish recovered in samples had moved into other nursery habitats in the bay. The only significant movements observed were from release habitats into the streams located immediately to the north of each release site. This pattern was repeated after spring and summer releases. There were isolated cases of fish moving from one release habitat to the other, as well as movement from

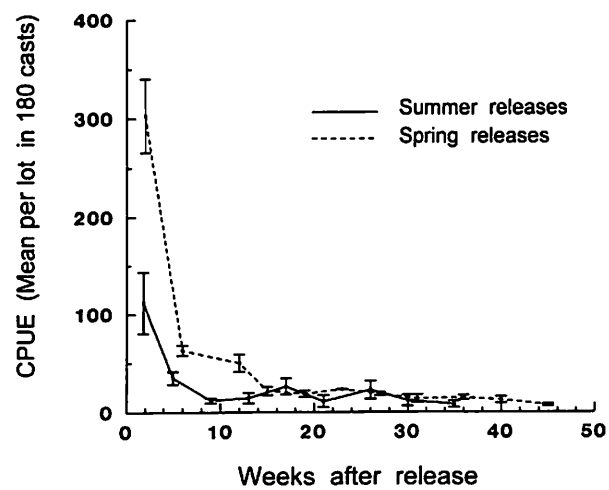

Figure 2. Mean number of tagged cultured fish in samples following spring and summer releases into Kaneohe Bay. Data are means per release lot (±standard error [SE]; n = 6 lots per season [3 at each release site]).[2]

release habitats into other nursery habitats in the bay. But the magnitude of dispersal out of release habitats and beyond the streams immediately north of those sites was negligible. Overall, 90.8%±3.1% (SE) of the cultured fish collected through 36 weeks in the wild were recovered at the nursery habitats into which they had been released.

Growth. Growth after spring releases was similar to growth following summer releases. Length increase following releases is plotted in Figure 3 for fish from the 70-85 mm treatment group, which was representative of all 5 size-at-release groups. There was little change in mean length during winter months (from September, 1991 through February, 1992; weeks 20-45 following spring releases in Figure 3).

Release Season Effect on Recapture Frequencies Among Size-at-Release Groups. Recapture frequencies ([number recaptured/number released] * 100%) within size-at-release intervals revealed an obvious and direct relationship between size-at-release and recapture rate (Figure 4) -- when fish were released in summer, recapture frequency was almost directly proportional to size-at-release within 1 month after release. This pattern was evident throughout the rest of the study. In contrast, size-at-release had much less effect on recapture frequencies for fish released 10 weeks earlier, in the spring (Figure 5).[2)]

Recapture frequencies of small tagged fish (<70 mm TL) were clearly greater throughout collections made following spring releases than in those after summer releases. After 45 weeks in the wild, fish from the smallest size classes released in spring remained abundant in net samples. The relative impact derived from the smallest fish released in spring (45-60 mm) corresponded to impacts of some of the larger sizes released. In contrast, on the majority of collection dates following summer releases, not a single individual released in summer was collected from the 45 to 60-mm size-at-release group. After a few months in the wild, the larger fish released (>85 mm) generally were more abundant in samples when they were liberated in summer rather than spring.

To compare recapture frequencies statistically among size-at-release intervals, values per release lot were summed across weeks for the period between 16 and 36

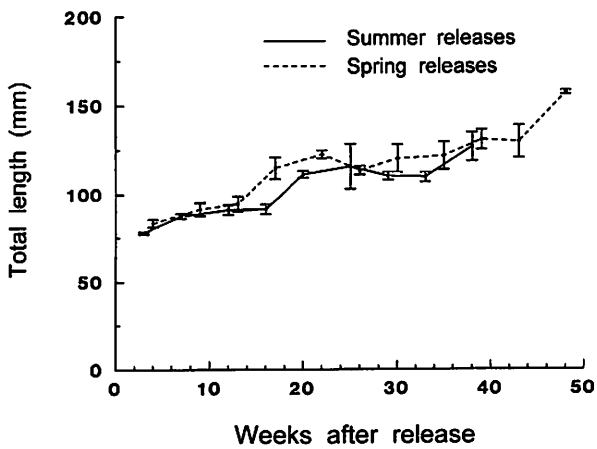

Figure 3. Mean total length (±SE) of cultured fish recaptured in collections made following spring and summer releases into Kaneohe Bay. Data are for the 70-85 mm size-at-release interval. Length was averaged within replicate release lots. Standard errors were based on replication established by release lots (n = 6 lots per season [3 at each release site], not total number of individuals recaptured).[2)]

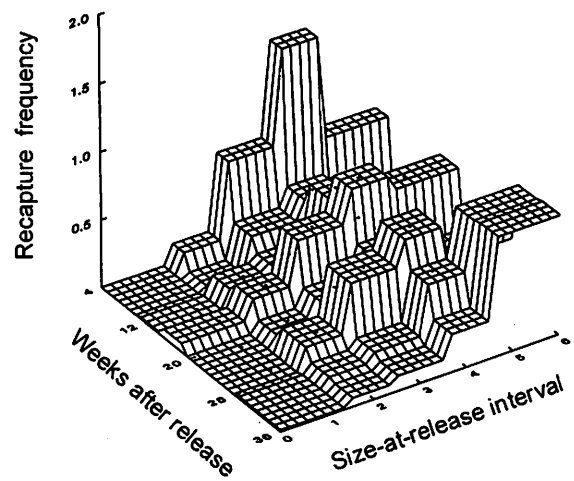

Figure 4. Recapture frequencies of tagged cultured *Mugil cephalus* recaptured in cast net samples after SUMMER releases into Kaneohe Bay. Data are presented for each of the five size intervals released (Size-at-release: 1=40 to 60-mm total length, 2=60 to 70 mm, 3=70 to 85 mm, 4=85 to 110 mm, and 5=110 to 130 mm). Data are percent recaptured of the total fish released per size-at-release interval.[2)]

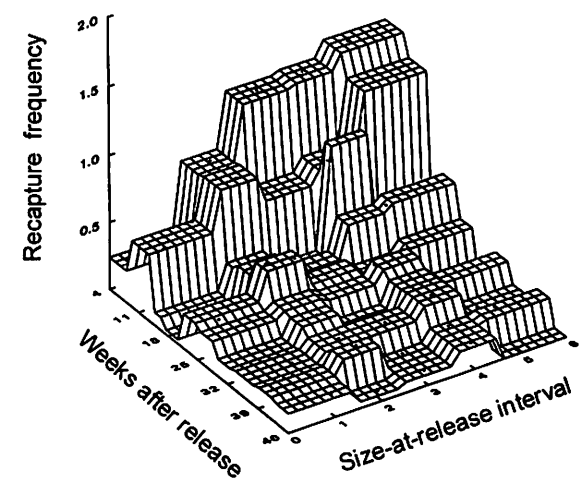

Figure 5. Recapture frequencies of tagged cultured *Mugil cephalus* recaptured in cast net samples after SPRING releases into Kaneohe Bay. See Figure 4 for description of fish size-at-release. Data are percent recaptured of the total fish released per size-at-release interval.[2)]

weeks after releases. After summer releases, mean recapture frequencies of fish <70 mm when released were substantially less than frequencies for fish >85 mm when released (Figure 6; ANOVA, $P<0.001$ in a posteriori orthogonal contrasts[41] of intervals 1 and 2 combined versus intervals 4 and 5 combined).

However, with the data from spring releases, mean recapture frequency of the smallest fish released (45 to 60 mm) was statistically similar to frequencies of some of the larger fish released (70 to 85 mm and those >110 mm) (Figure 7; $P = 0.33$). Fish from groups 2 and 4 (60 to 70 mm and 85 to 110 mm when released) had marginally greater recapture frequencies than those for small fish ($P < 0.03$; spring releases). Fish from the two largest size intervals (fish >85 mm) released in summer exhibited mean recapture frequencies about twice as high as those for any size fish from spring releases ($P<0.02$).

Interaction between size-at-release effects and release season effects was statistically significant ($P=0.01$, season x size interaction term). A significant interaction term indicates dependence of one factor upon the other; in this case, size-at-release affected recapture rate ($P<0.001$), but the degree of that effect depended upon release season.[2]

Test of marine stock enhancement potential in striped mullet nursery habitats.[25]

Recapture Summary

A total of 2,992 tagged cultured mullet were recaptured during the 11-month period of this study. Of these, 2,642 were cultured fish from the 1992 releases. None of the fish released in 1992 were recaptured at Kaneohe stream. Based on the tag codes, 304 (10.2%) of the 2,992 tagged fish collected had been released in Kaneohe Bay in 1991 as part of the 1991 study of release season effects.[2] Tags from 46 (1.5%) of the recaptured fish were lost during the extraction process. These 46 cultured fish were released either in 1991 or 1992, but could not be further identified and are excluded from analyses. Thus, the decoded tag data are based on 2,946 tags.

Tag retention in the subsampled fish held for 4 months averaged 97.9% (± 0.6 SE, $n=12$ tanks). With one exception (92.6%, lot 1, lagoon release), all tag retention rates within release lots exceeded 97%. Adjusting capture data for the 2.1% tag loss reveals that about 2,697 cultured fish from the 1992 releases were actually captured (3.3% of the 80,507 fish released in this study).

Tagged cultured fish comprised about 50% of the 5,708 wild and cultured mullet collected from the four study sites. Proportions of cultured mullet in Kahaluu samples remained $>50\%$ of the total (wild and cultured) mullet sampled in collections throughout the study, and were $>70\%$ in six of ten collections. Proportions of hatchery fish were also high at two streams north of the release site.

Recovery of Yearlings Released in 1991

Sample dates in the present study ranged from 47 weeks to 100 weeks after the 1991 releases. Most (86%) fish from 1991 releases were collected at Kaneohe Stream, where they comprised a high proportion of 1-yr old mullet at that site throughout their second year in the wild. Only 42 of

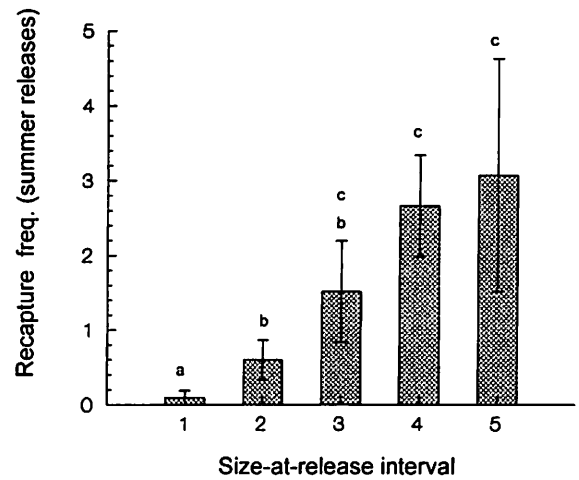

Figure 6. Mean Recapture frequencies ($\pm$SE, n = 6 lots) for the five sizes of fish released into Kaneohe Bay during SUMMER releases (see Figure 4 for description of fish size-at-release). Data are mean recapture frequencies per release lot ([number recaptured/number released] * 100%) summed over collections made between 16 and 36 weeks after release. See Figure 4 for description of fish size-at-release codes. Letters above bars indicate results of multiple comparisons of means; size-at-release intervals that share the same letter were not significantly different.[2]

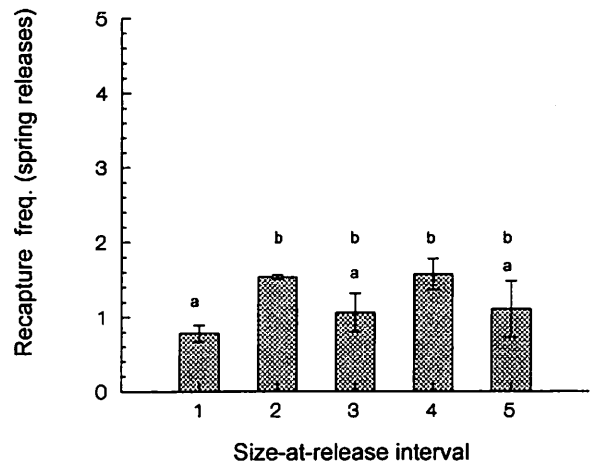

Figure 7. Recapture frequencies ($\pm$SE, n = 6 lots) for the five sizes of fish released into Kaneohe Bay during SPRING releases (see Figure 4 for description of fish size-at-release). Data are mean recapture frequencies per release lot ([number recaptured/number released] * 100%) summed over collections made between 16 and 36 weeks after release. Letters above bars indicate results of multiple comparisons of means; size-at-release intervals that share the same letter were not significantly different.[2]

the 304 fish from the 1991 release were collected outside Kaneohe stream. These 42 fish comprised <3% of the cultured fish collected at any other site. Thus, some fish from 1991 releases continued to occupy juvenile mullet nursery habitats well into their second year.

Evaluation of Marine Enhancement Impact: 1992 Releases

Release Impact in Nursery Habitats. About 90% of the 2,946 tags recovered and decoded were from striped mullet released at Kahaluu in 1992. These fish made a substantial contribution to juvenile recruitment in three nursery habitats in the north end of the bay: Kahaluu stream, Kaalaea stream and Waiahole stream.

Impact of the test release was greatest at the release site, Kahaluu stream; there was a trend towards reduced impact with shoreline distance away from that site. Cultured fish consistently outnumbered wild fish at the release site, and averaged 66% of the mullet in monthly collections at Kahaluu. After 11 months in the wild, cultured fish still comprised 50% of the mullet sampled at Kahaluu. Greatest impact outside of the release site was seen at Kaalaea, 1 km north of Kahaluu stream, where cultured fish averaged about 50% of the mullet sampled. A substantial effect was also apparent in Waiahole Stream, a mullet nursery habitat 3 km north of Kahaluu Stream, where cultured fish averaged 28% of the mullet sampled. Proportions of cultured mullet in collections were stable through time at all four nursery habitats sampled, until spring when annual recruitment of wild mullet began. Numbers of both wild and cultured fish from the 1992 year class declined in samples in spring at all nursery sites.

Release Microhabitat Effect on Enhancement Impact. Initial habitat selection was strongly affected by release microhabitat. There was greater dispersal away from the release site by fish released next to the inlet of Kahaluu stream than by fish released about 300 m upstream, in the lagoon (Figure 8). This pattern was similar following both spring and summer releases. Most fish released upstream remained in Kahaluu stream throughout the study, whereas most fish from the inlet releases moved to other nursery sites in the bay. This difference in dispersal patterns was statistically significant by the second collection date ($P < .003$, n=6 release lots per treatment [3 spring+3 summer]) and observed through mid February 1993 ($P < .01$ in collections 2, 4 and 7; $P < .05$ in 5, 6 8; ns in 1, 3, 9 10).

Recapture rates and growth rates were unaffected by release microhabitat. Growth curves for fish released at the inlet and lagoon were intermingled (Figure 9), as were plots of numerical abundances of cultured fish in collections (Figure 10). Statistical comparisons were non-significant (n=6, $P > .08$) for all collection dates.

Comparison with 1990 and 1991 Release Impact. There was substantially greater impact on juvenile abundances in Kaneohe Bay following 1992 releases than after pilot releases in 1990[23] and in 1991.[2] Proportions of cultured fish at Kahaluu 10 months after releases increased from about 3% following 1990 releases, to 10% after 1991 releases, to about 50% in the present study (Figure 11).

The general pattern following releases was similar in all years-an initial increase in proportions of cultured fish in samples, followed by a decline over the next year. But, there were two principal differences in 1992: (1) July releases in 1992 caused a considerably greater increase in abundance than in earlier studies with July releases; (2) the late-summer decline in abundance resulted in less reduction in release impact in 1992 compared to other years.

Recapture rates were disproportionately higher in this study than after releases in 1990 and 1991 (Table 1). Number of fish released at Kahaluu stream in 1992 exceeded the number released at that same site in 1990 and 1991 by 690% and 180%, respectively. Yet total number

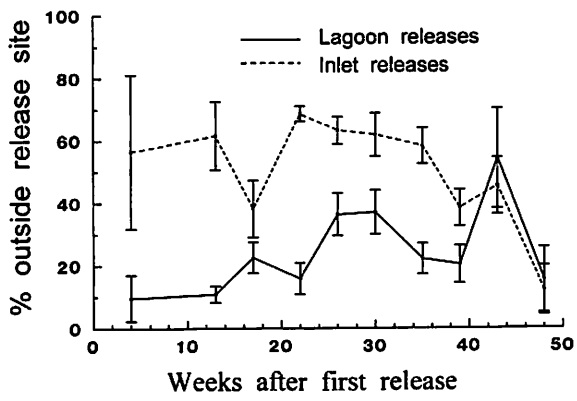

Figure 8. Release microhabitat effect on dispersal of released cultured fish. Proportions (±SEM, n=6 lots) of cultured striped mullet recaptured outside of the release habitat, Kahaluu Stream, following downstream (INLET) releases at the shore next to the stream mouth, and releases 300 m upstream (LAGOON).[25]

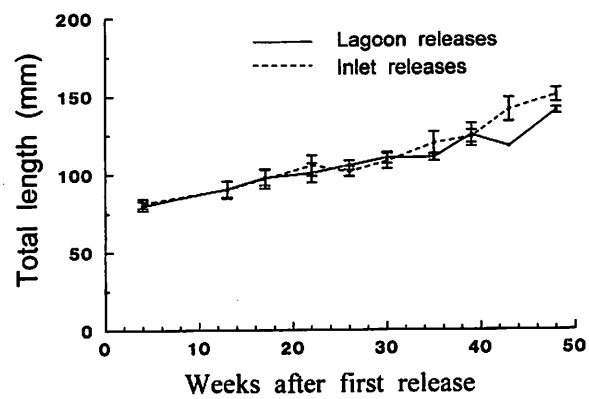

Figure 9. Mean length (TL; ±SEM, n=6 lots) of cultured striped mullet recovered in net samples made over the course of the study. All individuals were 70-85 mm TL when released.[25]

of fish recaptured from week 16 on exceeded comparable data (similar sampling effort) from 1990 and 1991 by 1560% and 420%, respectively (Table 1). Increase in the effectiveness of 1992 releases is revealed by comparing recapture frequencies (no. recaptured/no. released) and including data from all release sites. From week 16 on, 1.65% of the cultured mullet released in 1992 were recaptured in cast net samples. This recapture rate was 6 times that seen in the 1990 study (0.28%) and 1.7 times the rate in the 1991 study. Sampling frequency and number of cast-net samples were nearly identical among the three studies.

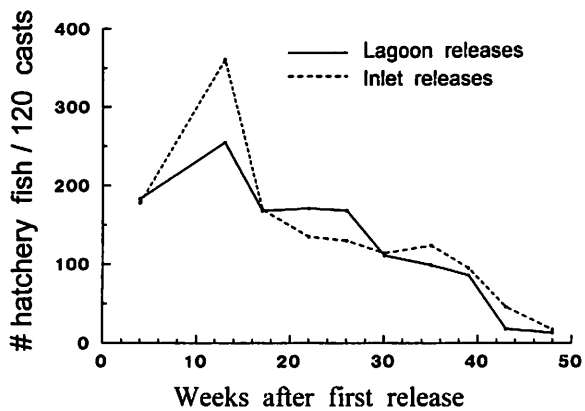

Figure 10. Catch per unit effort (CPUE) for cultured striped mullet from collections made at four nursery habitats (combined data) in Kaneohe Bay, including the release site. Total number of tagged fish recaptured in 120 cast-net samples monthly (30 samples per month at each nursery habitat) compared over the 11 mo study period.[25]

These differences among studies were statistically significant. Recapture rates after 1992 releases were significantly greater than expected in all pair-wise comparisons of 1992 data with data from 1990 and 1991 (Table 1; G tests, $P < .001$ in all cases). The 1991 study yielded significantly greater recapture rates than the 1990 study, when fish released outside of the Kahaluu site in 1990 are included in the comparison (Table 1; $X^2 = 225$, $P < .001$).

Effect of cultured fish on the striped mullet fishery: Survey of recreational fishers in 1992

A total of 130 survey trips were made at roadside and boat-ramp sites around Kaneohe Bay and Maunalua Bay between 15 August and 30 November 1992. Only six recreational mullet fishers were located and sampled, all on separate occasions. One fisher possessed two striped mullet taken with pole-and-line gear, and two had one striped mullet each, which were captured with a gill net and a cast net. All four fish were wild, and all were captured at

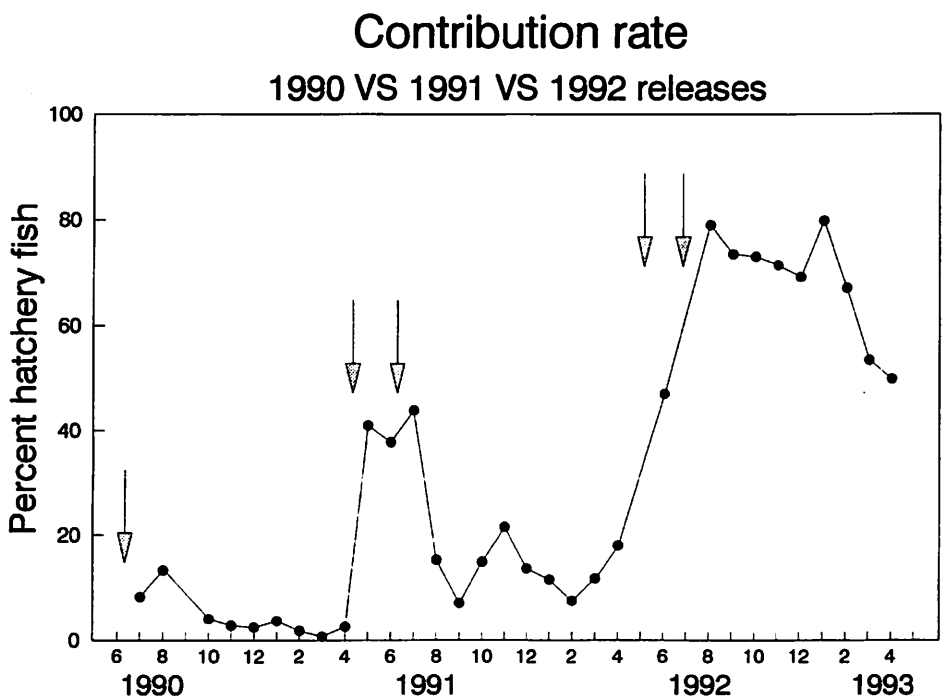

Figure 11. Percent contribution of cultured striped mullet to total abundance (wild and cultured striped mullet) in cast-net samples over a three year study period in Kaneohe Bay (including this study). Data are from monthly cast-net collections at Kahaluu Lagoon. Arrows identify release periods. Spring and summer releases were conducted in 1991 and 1992. Releases in 1990 were made only in summer.[25]

Table 1. Summary of numbers of cultured mullet released and recapture frequencies in Kaneohe Bay following hatchery releases in 1990, 1991 and 1992 (Leber et al., 1996). Chi-Square values (X^2) and probablity levels (P) from G tests are given for pair-wise comparisons of recapture frequencies (1990 vs 1991 and 1991 vs 1992). There were two release sites in Kaneohe Bay in 1990 and 1991.

	Kahaluu Stream			All Sites Combined		
Released	1990	1991	1992	1990	1991	1992
Total Tagged	11,676	45,790	80,507	42,822	91,245	80,507
Recaptured						
Total	177	952	2,632	227	2,405	2,632
% Recovered	1.52	2.08	3.27	0.62	2.64	3.27
X^2	16.0	148		689	56	
P	<.001	<.001		<.001	<.001	
Total After 16 wks.	85	315	1,326	118	890	1,326
%Recovered	0.73	0.69	1.65	0.28	0.96	1.65
X^2	.021	225		225	147	
P	<.640	<.001		<.001	<.001	

Wailupe Beach Park in Maunalua Bay.

The 1992 results indicated that Kaneohe Bay and Maunalua Bay had few pole-and-line recreational fishers. Because of the small sample size, an accurate CPUE estimate for wild striped mullet could not be obtained.

Effect of cultured fish on the striped mullet fishery: Modified survey targeting commercial fishers in 1993 and 1994

Survey participation. Over the two-year study period 181 fishing trips were sampled, including 107 surround net catches, and 74 gill net catches. Catch and effort data obtained directly from the field from surround nets and gill nets comprised 84.1% and 78.1%, respectively, of the total data collected for these fishing gears. The remainder was obtained indirectly through fish markets.

Contribution of cultured fish to the catch. During the two years that the modified creel survey was conducted, 4119 striped mullet were sampled from the commercial catch in Kaneohe Bay. Of these, 211 were tagged, cultured fish released in pilot studies from 1990-1993. In 1994, surround-net fishing yielded a mean of 7.22% (±4.32%) cultured fish per catch event, nearly double that during the same period in 1993. Cultured fish comprised 5.88% (± 3.85%) of the mullet caught in gill nets in 1994, compared to 0.71% (±0.37%) of the catch in 1993.

The data revealed a steady and significant increase in the percent contribution of cultured fish to the commercial striped mullet fishery during 1993-1994 (Figure 12; ANOVA, $P<0.001$, $F=9.07$, $df=103$). Contributions of cultured fish in 1994 were significantly greater than those in 1993 (separate-variances T-Test, $P<0.001$, $T=4.1$, $df=49.9$.[40]) By autumn 1994, cultured fish comprised 13.01% (±2.79%) of the commercial striped mullet catch in Kaneohe Bay, and this figure was increasing logarithmically.

Cultured striped mullet recovered from the fishery ranged in size from 270 mm TL (191 g) to 467 mm (906 g). Cultured fish entered the fishery as early as 59 weeks after release (270-mm individual, released in summer 1992). The next earliest entries were six cultured fish released in the summer of 1993, which averaged 340 mm in length (360 g) after 66-67 weeks in the wild, and which were all recovered in autumn 1994. The three oldest fish recovered were released in 1990, recaptured after 229 weeks, and averaged 377 mm TL (570 g). For samples of 20 or more fish, average growth ranged from 1.9 to 2.5 mm per week (Table 2). Growth rate decreased with fish age (Figure 13, $r^2=0.67$, $P<0.001$). There was considerable size

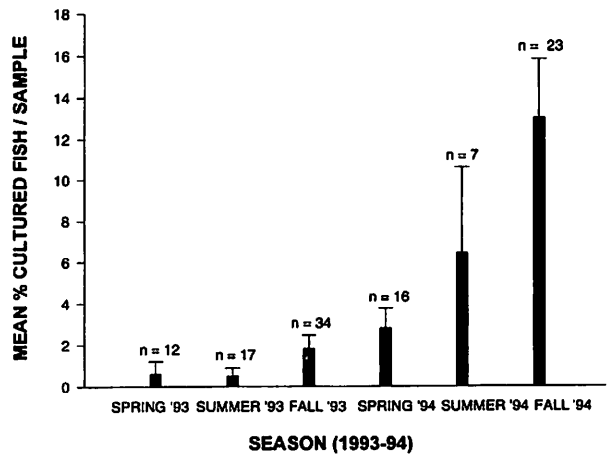

Figure 12. Mean percent contribution of cultured striped mullet in samples taken from the commercial mullet fishery in Kaneohe Bay. Sample size (n) = number of catches sampled in which striped mullet were landed. Fishing trips sampled where no mullet were landed are excluded.[32]

variation among SAR groups, and length was weakly correlated with weeks after release (Figure 14, $r^2=0.29$, $P<0.001$).

Effect of cultured fish on the striped mullet fishery: Release history and relative survival

Release year. Tag codes revealed that the majority (56.4%) of the 211 cultured striped mullet retrieved from the commercial fishery in Kaneohe Bay were released into the bay in 1992 at Kahaluu Stream (Table 2). Release history of one tagged fish could not be identified, because the tag was lost during extraction.

Recovery rates (number released/number recaptured) were significantly greater for fish released in 1992 than for those released in 1991 (ANOVA, $P<0.005$, n=12 separate releases each year). As expected because of the short time period after release, recovery of fish released in 1993 was low relative to recovery of fish released during 1990-1992.

Release habitat. Recovery rates were significantly greater for fish released at Kahaluu Stream in 1990 than along the shoreline at the Hawaii Institute of Marine Biology (HIMB) access pier (Table 2; ANOVA, $P<0.02$, n=2 release lots at Kahaluu and 3 lots at HIMB); the 31,146 fish released near HIMB pier apparently suffered complete mortality. There was also greater recovery from releases made in 1991 at Kahaluu Stream than from Kaneohe Stream ($P<0.001$, n=6 lots). Release habitat had no significant effect on recovery rates of fish released in 1992 at two locations within Kahaluu Stream (upstream lagoon versus stream mouth; $P>0.28$, n=6 lots).

Size-at-release and release season. Recovery rates were directly related to fish size-at-release (SAR), with dispro-

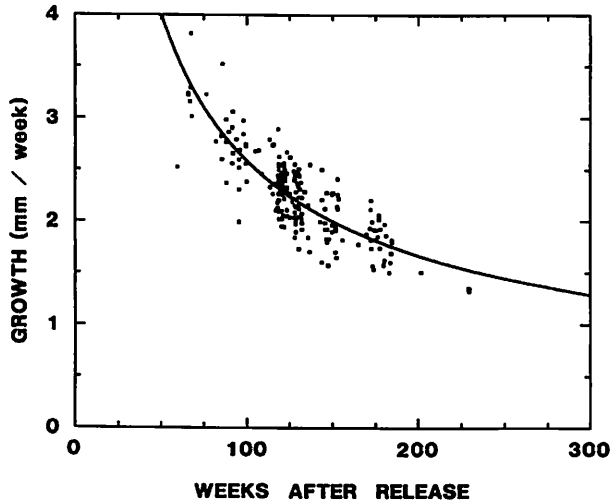

Figure 13. Decline in growth rate (mm total length per week) with age of cultured striped mullet recovered from the commercial fishery in 1993 and 1994.[32]

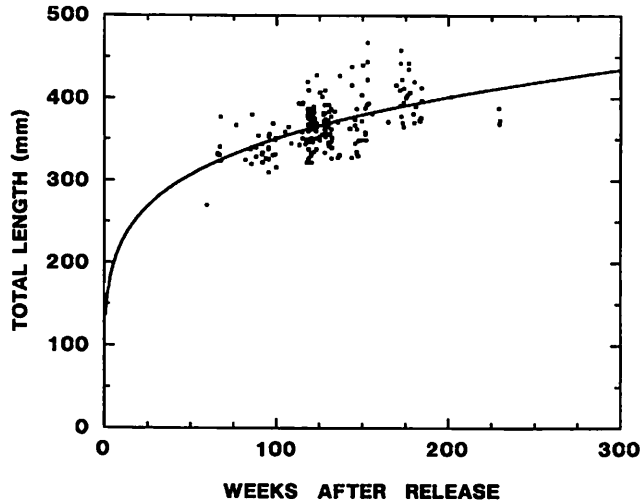

Figure 14. Total length (mm) of cultured striped mullet recovered from the commercial fishery.[32]

Table 2. Release study and release site of 210 cultured fish retrieved from the commercial fishery in Kaneohe Bay (Leber and Arce[32]). Abundance data are total number recovered and mean recovery rate (number caught/number released) per release lot for fish collected during creel interviews in 1993 and 1994. Numbers released and numbers of experimental replicates (Lots) per release site varied among years.

Release	Mean Release	Mean Number	Total		recovery		length		Growth	
Year	location	recovered	released	Lots	rate	SEM	(mm TL)	SEM	(mm x wk^{-1})	SEM
1990	Kahaluu Inlet	20	11,676	2	0.18%	0.05	410	17.73	1.90	0.20
1990	HIMB Pier	0	31,146	3	0.00%	--	--	--	--	--
1991	Kahaluu Inlet	55	45,362	6	0.12%	0.02	369	3.91	2.04	0.02
1991	Kaneohe Inlet	10	45,455	6	0.02%	0.01	399	6.47	1.88	0.09
1992	Kahaluu Inlet	68	40,223	6	0.17%	0.02	357	5.17	2.47	0.03
1992	Kahaluu Lagoon	51	40,284	6	0.13%	0.03	364	3.23	2.31	0.05
1993	Kahaluu Lagoon	6	29,354	3	0.02%	0.01	337	5.21	3.26	0.06

portionately low survival of fish that were smaller that 70 mm TL when released (Table 3). Recovery rates of fish larger than 85 mm when released were approximately 5 times greater than those for fish that were smaller than 60 mm when released.

SAR effect was strongly significant ($P=0.003$, n=14 replicate release lots) when data were pooled across years (including fish from releases in 1990, 1991 and 1992; but excluding fish from 1993 releases, as the largest fish released in 1993, 110-130 mm, were just beginning to enter the fishery at the end of the study period).

Cultured fish were released during different times of the year (release seasons) to evaluate the effect on survival of the timing of releases (spring and summer) relative to natural juvenile recruitment pulses (spring). The direct effect of SAR on recovery rate was strongest after summer releases (Figure 15, ANOVA, $P=0.011$, n=8 lots). Although not statistically significant ($P=0.20$, n=6 lots), there was a trend in the pooled data towards size-at-release dependent survival following spring releases (Figure 16). Compared to results from summer releases, however, there was better survival of fish below 70 mm when released in the spring. No fish were recovered from the smallest SAR group (45-60 mm) when releases were made in the summer (Figure 15). In comparison, 13 of the smallest fish released in spring (45-60 mm) were recovered in the fishery from the studies that included spring releases (1991 and 1992, Figure 16).

DISCUSSION

Importance of pilot release experiments to develop efficient release protocols

Fish size-at-release is clearly an important mediator of the effect of hatchery releases on stock abundance.[15, 16, 23, 30, 31, 33, 42, 43] At all of the release sites tested in Hawaii, size-at-release has been an important factor affecting recapture probability of cultured striped mullet.[23, 25] In previous studies with striped mullet, where releases were conducted in summer and fall, recapture rate was directly related to size of fish at the time of release.

As expected,[23] in the 1991 study, recapture rates after summer releases of small fish (individuals <60 mm long) approached zero and were an order of magnitude less than recapture rates of the larger fish released. Thus, when releases are made in summer in Kaneohe Bay, small (<60 mm) cultured striped mullet do not significantly affect juvenile recruitment in Kaneohe Bay. It is important to note that the fish in the different size intervals released were produced from multiple rearings, and that the smallest fish released in summer were not merely the slowest growing individuals; rather, size-at-release was related primarily to age.

A new finding revealed by this study was that the seasonal timing of striped mullet releases can substantially alter size-at-release effect on recapture rate. Compared to recapture rates after summer releases, recovery of the

Table 3. Numbers of recaptured cultured striped mullet organized by time in the wild and size groups released (Leber and Arce[32]). Time is given in quarter years. Recovery rate is number recaptured divided by number released x 100 percent. Cultured fish from each size interval were released annually from 1990 through 1992. At the time of release, fish ranged in age from three months old (45mm) to eight months old (130 mm). Only individuals greater than 70 mm TL were released in 1993.

Quarteryears atsea	Number recovered	Size-at-release (mmTL)				
		45-60	60-70	70-85	85-110	110-130
5	1	--	--	--	--	1
6	6	--	--	--	--	6
7	5	--	--	1	3	1
8	21	2	1	8	7	3
9	4	--	--	4	--	--
10	67	--	3	32	32	--
11	42	6	13	8	9	6
12	16	2	3	6	5	--
13	17	2	4	3	8	--
14	2	--	--	1	1	--
15	22	1	3	13	5	--
16	3	--	2	1	--	--
17	1	--	--	--	1	--
18	3	--	2	1	--	--
Total	210	13	31	78	71	17
Number released:	243,498	46,774	53,478	86,334	44,451	12,461
Recovery rate:	.086%	.028%	.058%	.090%	.160%	.136%

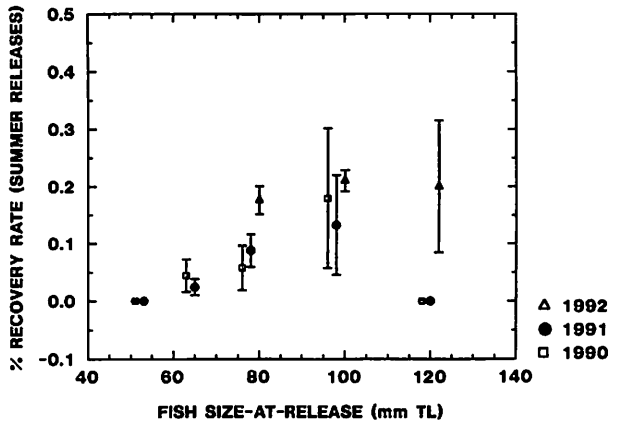

Figure 15. Mean percent recovery rate ([number recaptured/ number released] x 100%; ± sem, n=number of replicate release lots) of cultured fish in the commercial mullet fishery following summer releases into Kaneohe Bay. Data within release years are plotted against medians of the five size-at-release (SAR) intervals and offset around each SAR median on the x-axis for clarity. SAR intervals were 45-60 mm, 60-70 mm, 70-85 mm, 85-110 mm, and 110-130 mm total length.[32]

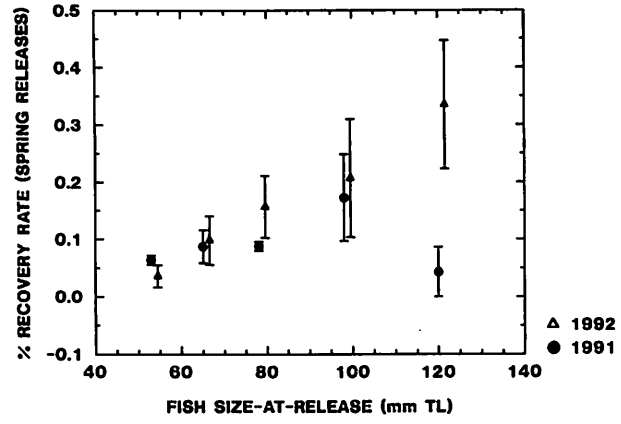

Figure 16. Mean percent recovery rate in the commercial mullet fishery following spring releases of five size intervals of cultured striped mullet into Kaneohe Bay. See Figure 5 legend for details.[32]

smallest individuals released was significantly greater when releases were timed to coincide with peak recruitment of small wild individuals (in the spring). This was the first evidence that releases of relatively small (45 to 60 mm TL) individuals could make any lasting contribution to striped mullet abundances in nursery habitats on Oahu. Subsequently, Leber and Arce[32] showed that some of the small fish released in spring did survive to adult size and contribute to the commercial fishery catch in Kaneohe Bay. The latter study also revealed that the smallest individuals in summer releases from this study apparently suffered total mortality. Because of the obvious economic importance of our findings, we replicated part of this study in a follow up study, with spring releases of the same size groups studied here; the results were identical -- small fish (<60 mm) did contribute to juvenile recruitment when releases were made in spring.[25]

Based on the 1991 study and on subsequent data on adult recruitment to the commercial fishery,[32] striped mullet <60 mm should not be released during summer in Kaneohe Bay. However, early (spring) releases of 45 to 60 mm striped mullet can make a contribution both to juvenile recruitment (this study) and to adult recruitment.[32] Maximum recovery from summer releases will occur when individuals are > 85 mm at the time of release. To determine optimal size-at-release, an economic analysis is needed to evaluate benefits and costs of releasing larger individuals.

Bilton et al.[31] showed an interaction between release timing and size of juvenile coho salmon, *Oncorhynchus kisutch*, released in British Columbia. In that study, returns would be maximized from early release of large juveniles. The effect of the seasonal timing of releases on size-dependent recapture rates may not be universal (e.g. Willis et al.[33]) ; nevertheless, release season could be a key factor in successful enhancement of many marine species.

What processes could account for the seasonal change in size-at-release dependent recapture rates? Size structures of cultured and wild fish suggested that schooling behavior of striped mullet may partly control the release-season effect. Schools of juvenile striped mullet are usually aggregated according to size.[23] Because of the difference in size structures between wild and cultured fish in the spring, after spring releases schools of larger striped mullet contained mostly cultured fish and few wild fish. We hypothesize that at the time of spring releases, the large individuals were more susceptible than were smaller ones to mortality from predation. We reason that, because the smallest fish released in spring had merged with relatively large numbers of small wild striped mullet, the smallest fish should have been afforded greater refuge from predators than were the large fish in our spring releases, which found few wild individuals their size (i.e. refuge effect from schooling behavior; e.g. Parrish[45,46] ; deVries[47] ; Ranta et al.[48]).

This pattern was reversed following summer releases, when size structures of the larger cultured and wild individuals were equivalent. By summer, most wild juveniles had grown larger than the size range of the smallest cultured individuals released. Thus, few small wild juveniles were available to form schools with small cultured fish. This should have reduced the advantage that refuge due to schooling behavior would provide small cultured fish.

The results of this study are consistent with the hypothesis that size-selective predation is a primary mechanism controlling recapture rates following hatchery releases in Kaneohe Bay.[23] Although, after summer releases, large

wild fish were not as abundant as small wild fish were in the spring (thus reducing the advantage gained by cultured fish from schooling with large wild fish), larger cultured fish would have the added advantage of size-escape from predation. Whatever the cause (s) of size-at-release impact on recovery rates, it was clear from this study that release season can influence the underlying mechanism.

The importance of conducting test releases to evaluate release strategies prior to conducting full-scale hatchery releases cannot be overemphasized. The 1991 study documented that release season can have a significant effect upon recovery of cultured striped mullet in the wild by affecting size-at-release dependent recapture rates. To optimize the impact of full-scale releases, marine stock-enhancement programs should perform test releases to evaluate interaction of release season with size-at-release effects.

We hypothesize that survival of cultured fish will be greater when releases are timed so that size-at-release coincides with modes in population size structures of wild stocks. A corollary to this is that the fewer cultured fish there are in a particular size interval at the time of release, the lower survival will be of wild fish in that interval.

These results need to be related to hatchery costs required to rear fingerlings to various sizes, and also to the increased production allowed by releasing small fingerlings in the spring, because this would make nursery tanks or ponds available to grow more fish for summer releases.

Although the mechanism underlying the direct relationship between survival and size-at-release is not well understood, it is clear that in Hawaii fish size-at-release can determine release success following summer releases. Based on this study, critical release size (CSAR, the size-at-release below which probability of survival approaches zero[23]) for enhancing striped mullet in Kaneohe Bay appears to be lower when releases are made in spring (CSAR $<$45 mm) than when releases are in summer (CSAR $>$ 60 mm).

Effectiveness of stock enhancement in augmenting juvenile recruitment of striped mullet

The 1992 study to evaluate the potential to increase striped mullet recruitment success using hatchery releases of juveniles revealed a substantial hatchery contribution in nursery habitats following releases of cultured mullet (Leber et al.[25]). Results of that study corroborate the first corollary of the marine stock enhancement concept, that released fish can survive, grow and contribute to recruitment. Released juveniles integrated with wild mullet at primary nursery habitats in Kaneohe Bay. Cultured mullet were abundant in samples on every collection date over the 11-months. Cultured fish showed linear growth; those released in May 1992 doubled in size within 48 weeks, with growth rates similar to wild striped mullet. The second corollary, that cultured mullet are not displacing wild mullet at the Kahaluu Stream release site, was experimentally evaluated and corroborated in a follow-up field experiment.[24]

Hatchery effect on abundances in nursery habitats was remarkable after adjusting release strategy to incorporate findings from pilot releases in Kaneohe Bay. Except for anadromous fishes, there are very few examples where hatchery releases revealed the potential to double juvenile recruitment success with a marine organism (e.g., Kristiansen and Sv sand[49] for cod; Kitada et al.[10] for flounder; Honma[11] for scallops). Cultured fish released in this study increased recruitment of juvenile striped mullet at the release site in 1992 by at least 100%. This large effect was partly a function of a poor recruitment year for wild fish, and was partly due to higher survival following summer releases, compared to survival in earlier studies.[2,23] Release impact on abundance was also considerable in streams 3 km away from the release site. Decrease in proportions of cultured fish in samples after 8 months coincided with the seasonal period (March) when yearlings begin to move out of nursery habitats into deeper water, and new recruits begin to arrive.[23, 36]

Results from two years of pilot releases were used to identify optimal release strategies for the 1992 study. Discontinuing releases near the Hawaii Institute of Marine Biology (HIMB) pier after 1990 resulted in a$>$300% increase in recovery rate in the 1991 study; the increase in recovery rate was compounded in 1992 by modifying SAR protocol as well (Table 1). By confining releases in this study to the vicinity of Kahaluu stream, and adjusting minimum SAR upwards to include only fish above 70 mm TL in summer, we achieved a 590% increase in recovery rate over the 1990 study, and a 170% increase over the 1991 study (Table 1: after 16 weeks).

This study provided new information on effects of release site. Choice of release microhabitat at Kahaluu (inlet and upstream lagoon) affected dispersal north from Kahaluu into other streams, but had no apparent impact on survival. The similar survival was surprising, given poor survival in 1990 of fish released along the shoreline near HIMB pier, relative to fish released at streams.[23, 32] We hypothesized that refuge from predators, afforded by mangroves and other shoreline vegetation in the north end of Kaneohe bay, accounted for the better survival of mullet released at Kahaluu inlet that dispersed along the shoreline.[25]

Release microhabitat affected the extent of enhancement in Kaneohe Bay in this study by partially controlling colonization of nursery habitats north of Kahaluu. If a management objective for full-scale releases was to have a portion of fish from each release lot disperse into adjacent nursery sites in the bay (e.g. in order to maximize use of

available nursery habitat), then releases at the inlet to KahaluuStream should achieve this. If stronger site fidelity were desired, releases farther upstream would result in lower dispersal during the nursery phase of the life cycle. Ability to affect which nursery habitats are selected by released fish, coupled with knowledge about recruitment success of wild fish, could be used to help prevent overstocking a particular nursery.

This study also showed that information from pilot releases is critical for managing full scale stock enhancement in coastal environments. Using results of pilot studies to modify release protocol caused a considerable increase in recovery rates and hatchery contributions to striped mullet abundance in their nursery habitats. Even before fish enter a fishery, data on relative survival of juveniles following pilot releases can be used to design effective release strategies.

The results of this study could be magnified were enhancement activities expanded to include other nursery sites in Kaneohe bay, provided sufficient habitat is available. Leber et al.[24] reported hatchery releases of cultured striped mullet at Kahaluu had an additive affect on population size. Kahaluu, Kaalaea, and Waiahole streams are primary mullet nursery habitats in the Bay; including the latter two with Kahaluu as release sites would increase stock enhancement effect in Kaneohe Bay.

Contribution of cultured fish to the commercial mullet fishery in Kaneohe Bay, Hawaii

Cultured fish from pilot releases in 1990-1993 made significant contributions to the commercial striped mullet fishery in Kaneohe Bay during 1993-1994. The substantial proportion of cultured fish in the bay-wide fishery in 1994 was surprising, given that these were pilot-scale releases of less than 100,000 individuals per year, and that released fish had direct access to the sea (striped mullet spawn in seawater,[35] and nearly all cultured fish recovered were within the size range of mature adults). There was apparently low emigration of cultured fish out of the bay.

A similar release impact was apparent in a recreational mullet fishery in Hilo Bay, where small-scale hatchery releases comprised around 20% of the annual catch (Leber, Sterritt Nishimoto unpublished data). The Hilo recreational fishery is almost entirely focused *within* an important striped mullet nursery habitat, which supports both juvenile and adult cohorts. The commercial fishery in Kaneohe Bay targets schools of striped mullet swimming throughout the bay. This study showed that cultured striped mullet released as juveniles could survive to maturity and recruit to the adult wild stock in habitats outside of juvenile nursery grounds.

These results indicate that relatively small-scale hatchery releases can make a significant contribution to local mullet fisheries in Hawaii. The data suggest that spawning stock biomass of the wild stock is quite low in Kaneohe Bay, and that releases of cultured juveniles could help increase adult population size.

This study showed that recovery rates identified during the juvenile phase of the life cycle[23,25] were a reasonably good indicator of the effects of release strategies on survival patterns of adults. Consistent with the results from studies of juveniles, this study of adults showed: 1. a direct relationship between size-at-release and recapture rate after summer releases; 2. higher recovery of individuals <70 mm when released in the spring, rather than summer, and zero recovery of fish <60 mm if released in summer; and 3. that release habitat had an important effect when fish were released away from the vicinity of their freshwater nursery habitats -- shoreline releases near HIMB pier resulted in very poor (zero) recovery rates compared to releases in the vicinity of streams, regardless of size-at-release.

Such information about how release strategies affect survival and recruitment of cultured fish to adult cohorts is clearly needed to plan effective stock enhancement programs.[3, 50, 51] These findings were consistent with other marine studies of SAR effects on recovery rates and survival (e.g. Tsukamoto et al.[15] ; Sv sand Kristiansen[16] ; Ray et al.[43] ; Willis Falls, Dennis, Roberts Whitchurch 1995) and release habitat effects on survival and dispersal (e.g. Stoner[19] ; Iglesias & Rodríguez-Ojea[14]).

If the effects of release strategies were not understood, at least two situations could have resulted in complete failure of hatchery releases in Kaneohe Bay. First, both the fishery and juvenile data sets showed a critical release size of 60 mm (the smallest size released that was subsequently detected in the fishery) following summer releases. Thus, summer releases of smaller juveniles or postlarvae have low probability of success.

Second, release site clearly regulates stocking success, as over 30,000 juveniles released outside of nursery habitats preferred by striped mullet (in three replicate lots in 1990) apparently suffered complete mortality. Hence, results from several release sites should be considered before selecting locations for full-scale releases. Pilot stock enhancement studies in other locations may benefit by expanding monitoring effort to include samples of juveniles (starting within a week or two after releases), if retrieving tags from fishers is the primary approach for examining enhancement effect. Information gained from juveniles could be used to refine release protocols even before released fish enter the fishery.

Results from this study prompt another recommendation, for stock enhancement programs that use internal coded wire tags. Even if fiscal limitations prevent having a structured net-sampling program to retrieve coded wire tag data, a single technician working as an occasional crew member or observer on board commercial fishing vessels may afford the means to monitor stock enhancement results. That approach worked well in this study, where

most tag data were retrieved, in effect, by engaging professional fishers to sample adults in the striped mullet population.

Based on the stock enhancement studies with striped mullet in Hawaii, marine stock enhancement appears to have high potential as an additional fishery management tool for Hawaiian coastal fishes. Hatchery releases of striped mullet could be used in conjunction with fishing regulations and habitat protection with the expectation that recruitment success of juveniles and adults would increase significantly in Kaneohe Bay. To ensure that stocks are actually enhanced by hatchery-release activities, information from pilot studies needs to be coupled with additional management considerations to provide a controlled approach to stock enhancement.[3, 50, 51]

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MARINE ENHANCEMENT PROGRAMS - IMPLEMENTATION PLANNING

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ABSTRACT

Due to limitations on land and water of the required quality, competition for space, environmental concerns related to rearing fish in protected waters, and the high costs and advanced technology associated with rearing fish in closed systems in offshore cages or net-pens, combining aquaculture and capture fisheries through marine fish enhancement programs is becoming an attractive option. Previous efforts, along with some that are ongoing, have ignored the potential impacts of large enhancement stocking programs on the biota of receiving waters. Future programs involving marine fish enhancement will have to be conducted in conjunction with studies that reveal how other organisms are affected. This effort will require a multidisciplinary approach and require some new thinking in terms of experimental design and the conduct of the required research.

INTRODUCTION

Interest in marine fish enhancement, which involves the release of captively reared species into the wild, has developed rapidly during the 1990s. The long-predicted peak in marine fish harvests[1] finally seems to have occurred.[2] Over the past few years there has been a leveling off in world fishery landings according to the Food and Agriculture Organization of the United Nations. New populations of traditional species are not being found and even species that have not been widely sought as human food or as a source of fish oil and meal are being exploited to or beyond the abilities of their populations to avoid decline.

Once the notion that capture fishery harvests would, in fact, peak at something approaching 100 million metric tons annually was accepted by fishery managers, there was a great deal of enthusiasm for meeting the increased demand through commercial aquaculture. While that approach continues to be championed and supports an industry responsible for nearly 20% of total fishery products marketed around the world today,[3] having grown in volume at the rate of over 9% annually between 1984 and 1992,[3,4] future expansion of traditional aquaculture is predicted to be slower than it has been in the past few years.[5]

Many prime aquaculture locations have already been taken. In some regions of the world, overproduction has already led to a situation known as self-pollution in which effluents from upstream aquaculture sites are causing environmental problems for those downstream. Competition for water between aquaculturists and a variety of other users is another major limitation. The National Research Council of the U. S. National Science Foundation, in its landmark study of mariculture potential, determined that significant increases in production, at least in the United States, would be possible only in closed systems and in systems established in the offshore environment.[6]

If natural fish and shellfish populations are being exploited at levels up to and in many cases beyond sustainability, and the potential for expansion of the foodfish aquaculture industry is limited, how can future demands for seafood be met? One answer that is being increasingly discussed involves enhancement stocking. To many it seems like a refreshingly new idea, though enhancement efforts with marine fishes were made in earnest during the late nineteenth and early twentieth centuries in Europe and North America. Enhancement programs can be considered a form of ocean ranching except that rather than returning to the hatchery for capture, surviving stocked animals may eventually recruit into traditional capture fisheries.

HISTORICAL PERSPECTIVE

Overfishing is not a new phenomenon. There were concerns that the ocean off France was depleted as early as the mid-1800s[7] and both Europe and North America were

becoming increasingly aware of depletions in certain marine stocks by the late nineteenth century.[7,8] A Norwegian biologist, G.O. Sars, is credited, in 1866, with being the first to successfully spawn marine fishes. His successes were followed nearly 20 years later by the work of another Norwegian, G. M. Dannevig who established a hatchery for the production of cod. His son, Harald Dannevig, established a plaice hatchery in 1893.[7] Together, Sars and the Dannevigs were the pioneers of European marine fish culture.

The naturalist Spencer F. Baird, concerned about overfished populations of fish along the Atlantic coast of the United States, was successful in his attempt to have Congress create a federal agency to deal with the overfishing problem. In 1871 the U. S. Fish and Fisheries Commission was born and Baird became its first head.[8] Baird, who was responsible for establishment of the Woods Hole Oceanographic Institution, among other accomplishments, was dedicated to establishing hatcheries for the production of fishes for release. To that end, he engaged the services of the majority of the few fish culturists who were active at the time. Among those people were Seth Green, Livingston Stone, and Charles G. Atkins.

Species that were spawned by U. S. Fish and Fisheries Commission hatcherymen during the early years of the agency's existence included Atlantic cod (*Gadus morhua*), brook trout (*Salvelinus fontinalis*), lake trout (*Salvelinus namaycush*), rainbow trout and steelhead trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*), chinook salmon (*Oncorhynchus tshawytscha*), lake whitefish (*Coregonus clupeaformis*), largemouth bass (*Micropterus salmoides*), walleye (*Stizostedion vitreum vitreum*), yellow perch (*Perca flavescens*), American eel (*Anguilla rostrata*), American lobster (*Homarus americana*), and American oyster (*Crassostrea virginica*). The railroads provided space in baggage cars for fish and the personnel who tended the aquatic animals. The Commission had several specialized fish hauling railroad cars constructed which crisscrossed the nation for several decades. Atlantic salmon were introduced to the west coast and Pacific salmon to the east coast and even stocked in the Gulf of Mexico. American shad (*Alosa sapidissima*) were successfully established on the west coast, as were striped bass (*Morone saxatilis*). Pacific salmon (*Oncorhynchus spp.*) were successfully introduced to the Great Lakes in the 1980s but were never established along the Atlantic coast.

Not millions or hundreds of millions, but hundreds of billions of fish and shellfish were hatched and released around the nation by the U.S. Fish and Fisheries Commission. Hundreds of thousands of animals were also shipped to other countries. Rainbow trout and chinook salmon, for example, became established in New Zealand as a result of shipments made by the Commission. Brown trout (*Salmo trutta*) were introduced from Europe, as were common carp (*Cyprinus carpio*). Carp were highly thought of by Baird, though his view is not widely shared today in the United States.

The stocking of hundreds of millions of fish almost exclusively involved newly hatched animals. Hatcherymen (and indeed, they all were men at the time) were quite innovative in learning how to captively spawn a variety of fish and shellfish species. Some of their techniques were lost to present day aquaculturists and have been reinvented, but many were recorded in the annual reports published by the Commission. Somewhat surprisingly, the basic techniques have not changed in many instances, even in the face of astonishing improvements in technology.

Releases of newly hatched animals were necessary for many years because of the inability of hatcherymen to provide suitable first foods (a problem that persists with some species to this day). Prepared feeds for fish such as trout, salmon, and catfish - all with large eggs - were developed relatively early on, but feeding marine fish larvae was a problem that could not be resolved by the early aquaculturists. Thus, the larvae were stocked. There is virtually no evidence that any of the massive stocking efforts gave rise to improved fisheries, though recovery did, in some cases, occur. Recoveries may very well have been due to the imposition of regulations on fisheries, not to enhancement stocking efforts. It is more than likely that the stocking programs provided planktonic food for larger fishes.

Within several years after creation of the Commission, stocking activities were initiated by the states. Concentration ultimately was placed primarily on enhancement of recreational fisheries by both the state and federal government hatcheries. Also, attempts to spawn and stock marine fishes were abandoned with the exception of anadromous forms (salmon, steelhead trout, striped bass). All effort was placed on species that could be reared to at least fingerling sizes on the theory that the larger fish would have some reasonable opportunity to survive after stocking. The list of species that met that criteria increased over the years, primarily with the addition of freshwater fishes. The approach of stocking fingerlings has continued to the present, and there is currently increasing attention being paid to producing marine fish for enhancement purposes.

In 1993, in response to a law passed by the legislature in the state of Washington which instructed the Washington Department of Fisheries to begin producing fish to enhance depressed marine recreational fisheries, the Washington Sea Grant Program hosted a Marine Fish Culture and Enhancement Conference.[9] The purpose of the conference was to bring people who were currently involved or interested in marine fish enhancement to compare information and determine how such a program might be developed for Puget Sound, Washington. Information was presented on activities in Japan, North America, Norway,

and the United Kingdom. The most active program in the United States is being conducted with red drum (*Sciaenops ocellatus*) in Texas. Over the 21 years the program had been in existence through 1993, over 115 million fingerling red drum had been stocked by the Texas Parks and Wildlife Department.[10)]

PLANNING FOR ENHANCEMENT STOCKING

Historically, efforts to enhance marine fisheries have been conducted with only an eye on stocking large numbers of fish or, in more recent times, significant numbers of fish of sufficiently large size to have a reasonable probability of surviving to ultimately recruit to the fishery. There has been little or no interest in evaluating what the impacts of such stocking programs might have on the existing biota. The underlying assumption has been that if a population has been overfished or reduced for some other reason, the ecosystem will be able to readily accommodate the reintroduction of replacement fish. That assumption needs to be re-examined as it is undoubtedly oversimplistic.

There is ample evidence from freshwater fish stocking programs to support the conclusion that enhancement has many ramifications on the involved ecosystem. Stocking predators in ponds, lakes, and reservoirs commonly leads to stunting and poor production unless a proper forage base is maintained. One of the best examples in the United States involves attempts to maintain balanced fish populations in bass-bluegill ponds. Balancing the forage base against the predator population is particularly difficult when fishing pressure is applied. Overfishing on the predator (largemouth bass) may lead to overpopulation and stunting of the forage species (most often, bluegill, *Lepomis macrochirus*). Ultimately, it may be necessary to exterminate all the fish and start over if balance is to be achieved. That option is not available in the marine environment.

While inland fishery managers often concentrate their effort toward resolving the problem of balancing the forage and predatory fish species to obtain rapid growth and subsequent early recruitment, other components of the ecosystem are also impacted by fish stocking. Species other than sunfish may either be stocked as forage - examples being gizzard shad (*Dorosoma cepedianum*), threadfin shad (*Dorosoma petenense*), and minnows (family Cyprinidae) - or may be present as a result of natural invasion. All of the forage species, and the bass as well, feed on such lower trophic levels as the benthos and zooplankton. Thus, it is necessary to maintain healthy communities of those organisms. Predaceous insects, along with other benthic organisms and zooplankters, feed within their own communities, which also include herbivorous organisms. Phytoplankton needs to be available for the herbivorous filter-feeders, while grazers need benthic algae. Aquatic plants provide substrate for the *Aufwuchs* community and also provide food directly to some species.

As a result, the simple bass-bluegill pond is a very complex environment that is subject to change in conjunction with the population dynamics of the organisms contained therein. While change can be, and often is induced as a result of fishing pressure, alterations in the physical and chemical environment also result in responses by the biota. Abiotic changes can occur (examples are alterations in temperature, salinity, clay turbidity), and the biological community can impart changes on the physicochemical environment (influencing, for example, dissolved oxygen, ammonia, nitrite, nitrate, phosphate, plankton-related turbidity).

Let us suppose that through hard work, and not just a small amount of luck, we have succeeded in bringing a bass-bluegill population into balance and are capable of maintaining that balance through our management activities. Now, let us assume the owner of the pond decides that what is needed is a doubling of the predator population. To achieve that end the decision is made to stock fish; e.g., we elect to initiate an enhancement program. It is inconceivable that such an action can be taken without having ramifications throughout the pond community since the predator-prey balance will be disrupted. To provide sufficient feed for the predator, the owner may next have to stock additional forage fish, which will increase the pressure on the the benthos and/or plankton community and will also require more oxygen, increase the amount of ammonia being generated, and so forth.

It seems reasonable to assume that establishment of a marine fish enhancement program will have ramifications in the marine ecosystem similar to those that occur in freshwater situations. The stocking of large numbers of fish can be expected to lead to repercussions throughout the ecosystem. Those repercussions may be trivial, but they could also be severe.

Management of inland recreational fisheries often involves stocking a new water body or restocking a water body that has been rehabilitated (i.e., all the fish originally present were eliminated before restocking occurs). In either case, the composition of the final fish community is known. Fingerlings are stocked and allowed to grow for a period of one or more years before fishing is initiated. Good managers can maintain balanced populations for at least some period of time in a number of ways. Included might be the imposition of slot limits, encouraging or requiring catch-and-release, and engaging in supplemental stocking of predatory and/or prey species.

In large lakes and streams, it is usually not feasible to eliminate fish communities prior to stocking, nor is it desirable if those waters contain fish that may represent genetically distinct populations or are threatened or endangered. Management of such waters tends to be more difficult than management of small ponds and lakes. The situation in large freshwater bodies and streams is

comparable to that which exists in the marine environment, with the exception that fishing pressure in United States freshwater bodies is almost exclusively recreational. In the marine environment, both commercial and recreational fishing are practiced. Some forms of commercial fishing not only remove large numbers of target species, they produce bycatch that is usually discarded and may, as in the case of trawling, alter habitat.

Much of the interest in marine fish enhancement that has developed in the United States has focused on providing more fish in recreational fisheries. However, in many instances, the species being stocked or considered for stocking are of interest to both recreational and commercial fishermen. Red drum enhancement in Texas began before commercial fishing for that species, along with the spotted seatrout (*Cynoscion nebulosus*), was banned in 1981.[11] Between 1975 and 1986 over 96 million red drum eggs, fry, and fingerlings were stocked.[12] The program now involves only fingerling stocking. Texas is currently considering enhancement of croaker, *Micropogonias undulatus* (S. Holt, University of Texas Marine Science Institute, Port Aransas, pers. commun.), a major species in the bycatch of the commercial shrimping industry, but one that has limited appeal to recreational fishermen because of its small size. There is some interest in selling live croaker as bait and in deboning them for use in various prepared fish products.

Striped bass, an anadromous species, has been the subject of enhancement efforts along the middle Atlantic coast of the United States where all fishing for the species was curtailed to allow for possible natural recovery of the overfished population. Recovery was also stimulated through an enhancement program. Within a few years there was sufficient recovery to allow a return to fishing, both recreational and commercial.

Research on summer flounder, *Paralichthys dentatus*, culture aimed toward commercial culture and enhancement, is underway on the Atlantic coast of the United States as well. In California, a program aimed at evaluating the economic feasibility of hatching and culturing marine species for release as fingerlings was developed by the California Department of Fish and Game in 1984. The program has targeted white sea bass (*Atractoscion nobilis*) and California halibut (*Paralichthys californicus*) for enhancement.[13]

In the state of Washington, preliminary work on the culture of Pacific halibut, *Hippoglossus stenolepis*, has been undertaken to assess both the potential of the species for commercial aquaculture and enhancement.[14] The Washington State Legislature passed legislation in 1993 requiring the Washington Department of Fisheries to engage in the artificial propagation of marine fishes to enhance depressed recreational stocks of Pacific halibut, lingcod (*Ophiodon elongatus*), Pacific cod (*Gadus macrocephalus*), and rockfishes (*Sabestes* spp.).[9] Research on the survival of striped mullet (*Mugil cephalus*) releases and the impact of such releases on the wild population has been underway in Hawaii since 1989.[15]

As interest in marine fish enhancement expands, the need for developing the proper approach to the activity becomes increasingly apparent. Components, each of which was deemed to be "an essential aspect of a responsible approach to controlling and optimizing enhancement," have been stated as follows: [15]

1. Have a process for prioritizing and selecting target species.
2. Develop a species management plan that identifies harvest opportunity, stock rebuilding goals, and genetic objectives.
3. Use genetic resource management to prevent inbreeding and outbreeding depression.
4. Use disease and health management.
5. Consider ecological and life-history patterns when forming enhancement objectives and tactics.
6. Identify released hatchery fish and assess stocking impact.
7. Use an empirical process for defining optimum release strategies.
8. Define quantitative measures of success.
9. Identify economic and policy guidelines.
10. Have a process for changing production and management objectives and strategies based on stocking impact.

There are two camps with different points of view with respect to how marine fish stocks should be managed. One would favor protection and restoration of habitat in the absence of enhancement stocking, while the other would support enhancement stocking as one among many tools used in fishery restoration and management. If the latter approach is taken, perhaps in conjunction with a habitat protection and restoration program, the above 10 items need to be taken into consideration. None of them, with the possible exception of number 6, seems to bear directly on how enhancement might affect other components of the biota. The identification portion of item 6 involves tagging hatchery fish in order to distinguish them from wild fish. The concern in that case involves the potential displacement of wild fish by hatchery fish of the same species.

In a survey of selection criteria for species suitable for use in enhancement programs, effects of such programs on the resident biota ranked 10th out of more than 25 items. More highly ranked items involved economics, the level of available technology, and the likelihood of success in increasing the population of adults.[15] It seems probable that those surveyed were largely individuals interested in developing enhancement programs and not those involved in maintaining and restoring habitat. When the subject of marine fish enhancement is discussed around environmental scientists, concern about impacts on other components of the biota is typically a top priority.

It seems logical that any marine fish enhancement program developed in the future should include a long-term research component that addresses the impacts on resident biota. Each program should include pre-stocking studies that characterize the ecosystem to be stocked in terms of the quality and quantity of each species present in the various trophic levels, nutrient and trophic dynamics, and population dynamics of the economically important species. Predictive models of ecosystem response to various stocking scenarios should be developed to assist in the decision-making process prior to implementation of the enhancement activity. Monitoring of the situation should coincide with enhancement stocking and the program should employ the adaptive management approach which will provide the flexibility needed to respond to changing conditions relative to the biota. Of critical importance are changes in predator-prey relationships that may occur with the implementation of enhancement stocking.

Studies of the nature described suffer from a lack of controls. True replication is virtually impossible, though similar coastal areas - one stocked and one unstocked - could be compared by conducting parallel studies. The level and type of fishing activity should be as similar as possible, so regulatory agencies would have to conduct or cooperate in the research. Monitoring of the stocked fish should include not only measurements of survival and recruitment, but also the extent of migration from the target area. Complete life history information will provide researchers with a considerable amount of insight into not only migratory habits but also food habits, fecundity, potential growth rates, and age at recruitment.

Clearly, enhancement programs should be developed in a manner which involves the formation of interdisciplinary teams of scientists, engineers, and technicians to produce the fish, stock them, and conduct pre- and post-stocking assessments. Regulatory and management agencies will also be integral to such teams, as will educators and extension specialists who can keep the public informed throughout the process.

From the layman's point of view, a successful program might be one in which large numbers of fish are continuously stocked, year after year. Success will be judged on the basis of whether the fishery provides the angler or commercial fisherman with a satisfactory supply. However, if enhancement of one species is successful at the expense of other species, whether those others are of economic importance or not, the program cannot be termed successful. That determination cannot be made in the absence of long-term monitoring. At some point, undoubtedly reached only after a long period of manipulation of stocking rates, regulation adjustment, and study, an equilibrium condition might be reached or predictive models sufficiently developed to have a high degree of reliability. At such times research activity may be reduced or curtailed, though it must be acknowledged that further research may be needed in the future when the equilibrium is disrupted, as is almost certain to happen.

Existing enhancement programs are of the type where fish are added to the ecosystem and results are evaluated largely on the basis of whether the target fishery improves. In the future it will be necessary to take a much more deliberate approach and document that enhancement efforts are not detrimental. That type of documentation can only be made if a properly designed research program is implemented. Such programs will add significantly to the expense associated with enhancement stocking programs, but should be considered essential.

The world capture fishery appears to have peaked and suitable land and water for confinement aquaculture are becoming increasingly scarce, while the human population, and thus demand for fishery products, continues to increase. Thus, new approaches are needed. Moving offshore and into closed water systems are two approaches that have been advocated,[6] but neither is without significant limitations. Offshore systems are very expensive and require innovative engineering. Closed systems have improved greatly in recent years but there continue to be few economically viable systems in operation.

Marine enhancement stocking programs could become common in the future. It is an activity that would link aquaculturists with commercial fishermen. The two groups are currently in conflict in many U. S. fisheries, but both could profit from properly designed and operated enhancement programs. Initially, such programs may be restricted to nonmigratory coastal species, but there is no reason that they cannot be extended to pelagic migratory fishes such as tuna (*Thunnus* spp.) and dolphin (*Coryphaena hippurus*). Such efforts would increase the stakes by requiring the cooperation and collaboration among all nations that target the enhanced fishery. While there are many problems to overcome, there is every reason to believe that if marine fish enhancement ever reaches its full potential it could become a major source of seafood worldwide.

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IMPACTS OF BIVALVE INTRODUCTIONS ON MARINE ECOSYSTEMS : A REVIEW

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ABSTRACT

The introduction of exotic or non-native bivalve species to coastal communities is appealing from an economic standpoint if it results in a new fishery, augments an existing fishery, or provides diversification for a mariculture industry. Introductions of exotic species can potentially affect local ecosystems in both negative and positive ways. Negative impacts from introductions result from three main sources: introduction of pathogens; introduction of "hitchhiker" species;and biological competition (including genetic influences) with native species, resulting in a loss of biodiversity. Conversely, an introduction may have a positive impact on an ecosystem by providing a missing component in a food chain that has been lost due to overharvesting or disease. This review synthesizes available knowledge on this topic and examines research conducted in response to the proposed introduction of an exotic bivalve to Chesapeake Bay (USA). Suggestions for minimizing negative impacts from future introductions are provided.

INTRODUCTION

Throughout human history, plants and animals have been moved from one part of the world to another. According to Hedgpeth[1], it was the policy of the U. S. Fisheries Commission to "populate the waters of the U. S. with as many useful or valuable food species as possible". Oysters have probably been moved from place to place more frequently than any other marine animal.[2] It is now recognized that the transportation of species beyond their historical geographical range, regardless of the mechanism involved, can have dire ecological consequences.[3]

Nonetheless, there is strong economic incentive to continue intentional introductions of exotic or non-native species to local waters. Total harvest of world fisheries is presently close to the estimated sustainable harvest of 100 million metric tons per year.[4] In addition, world population is growing by approximately 86 million persons per year, and per capita consumption of seafood is increasing.[5] To fill this escalating gap between fisheries harvest and demand for seafood, aquaculture production, which now provides about 19% of the world fish supply, will have to double by 2010 to meet global seafood needs.[6]

In coastal areas, introductions of commercial marine species have been undertaken intentionally to augment (or replace) depleted natural stocks and to diversify aquaculture operations. In many communities, aquaculture is becoming a viable economic alternative to captive fisheries.[7] Publically supported aquaculture is viewed as a means of augmenting natural populations (stock enhancement) which will result in economic gain. Commercial (private) aquaculture is seen as a source of jobs and long-term economic growth. Both of these approaches have the added benefit of reducing harvest pressure on existing stocks.

This review attempts to synthesize information regarding known (intentional) introductions of marine bivalves (summarized in Table 1), paying particular attention to impacts on local ecosystems. As such, it updates and is broader than previous papers describing introductions of exotic oyster species[8] and disease risks associated with introductions of exotic marine animals.[2] In addition, research conducted in response to a proposed introduction of Pacific oysters, *Crassostrea gigas*, to the east coast of the United States is summarized. Finally, conclusions regarding introductions of exotic bivalve species are provided.

NEGATIVE IMPACTS OF INTRODUCTIONS ON LOCAL ECOSYSTEMS

Introductions of exotic species of marine bivalves can have three main negative impacts on local ecosystems. These include the introduction of parasites and pathogens, the introduction of "hitchhiker" species, and biological (including genetic) competition with native species which results ultimately in a loss of biodiversity.

Table 1. List of documented introductions of marine bivalves discussed in this review

Species	Year	Site of Origin	Site of Intro.	Source
A. irradians	1979	Connecticut (U.S.)	Canada	33
C. angulata	1869	Portugal	France	18
C. gigas	1902-19	Japan	Washington (U.S.)	8,40
C. gigas	1912-78	Japan	B.C., Canada	41,42
C. gigas	1966	Japan	France	19
C. gigas	1971-75	B.C., Canada	France	18,19
C. gigas	1971-77	Japan	France	18,19,23
C. gigas	1947-52	Japan	Tasmania	47,48
C. virginica	1869+	eastern U.S.	W. coast U.S.	1,8
C. virginica	<1900	eastern U.S.	Great Britain	8
C. virginica	1900-02	eastern U.S.	Washington (U.S.)	8,40
C. virginica	1903-36	eastern U.S.	B.C., Canada	41,42
C. virginica	1914	eastern U.S.	P.E.I., Canada	8,9
M. mercenaria	1869+	eastern U.S.	W. coast U.S.	1,8
O. edulis	1949-61	Netherlands	eastern U.S.	28
O. edulis	1963-65	Connecticut (U.S.)	California (U.S.)	29
O. edulis	1970s	California (U.S.)	France	26,27
O. edulis	1957-59	United Kingdom	Canada	44

Introduction of Pathogens and Parasites

Introduction of exotic bivalve species provides an opportunity for the introduction of pathogens and parasites into susceptible populations. According to Sindermann[2] there are three categories of disease risk associated with introductions. These include (1) risk from known pathogens of the introduced species, which may or may not be transferred to the local population; (2) risk from organisms having unknown pathogenicity to the introduced species, but possibly pathogenic to the native species; and, (3) risk from other organisms, rare or unrecognized, in the introduced species that may be pathogenic to related native species. Although it is difficult to establish that a particular disease agent has been introduced along with the exotic host, there have been several instances of shellfish introductions that preceded major epizootics in native populations without appreciably affecting the introduced species.

Malpeque Bay Disease in *C. virginica* (Canada)

Crassostrea virginica was introduced to Prince Edward Island, Canada, from New England in 1914 to supplement reproduction in local stocks which had been depleted from overfishing.[8] Severe oyster mortality first occurred in 1915-16 and continued throughout the island until about 1930. Surviving oysters propagated, and mortality resistance was developed over several generations. The causative agent of this epizootic was never identified, and never caused mortality in New England, from where it supposedly originated.[8] The disease agent may still be present, however, as nonindigenous oysters are susceptible and die within 2 yr.[9]

MSX Disease in *C. virginica* (USA)

Massive mortalities of oysters, *C. virginica*, were first observed in Delaware Bay in 1957 and in Chesapeake Bay in 1959.[10] In both estuaries, over 90% of oysters growing at salinity > 15 ppt were killed within 2 yr. A previously unidentified protozoan parasite, *Haplosporidium nelsoni*, was linked to these mortalities.[11] Since the original epizootic in 1957, *H. nelsoni* has been found as far south as Florida and as far north as Maine, where an epizootic recently occurred as the result of unusually warm and dry climatic conditions.[12]

Andrews[8] speculated that *H. nelsoni* originated from Asia and was carried to the east coast of the United States with importations of exotic oysters. Many small introductions of exotic oysters were made along the coast, most of them undocumented.[8, 13] A bushel of *C. gigas* was planted in Barnegat Bay, New Jersey in the early 1930's but failed to grow and died within 2 yr. An oyster grower from Delaware had some *C. gigas* shipped to Delaware Bay after seeing some at the Seattle World's Fair in 1962. These oysters were confiscated by a biologist who held them in open waters for several years without serious mortality or apparent successful reproduction. *Crassostrea gigas* was also planted in Maryland waters by a seafood dealer; this resulted in a specific law in that state prohibiting the species.[8]

Even though none of these introductions of *C. gigas* to the east coast of the United States corresponded directly in time with the sudden outbreak of MSX disease in Delaware Bay, it is well established that *C. gigas* had been introduced to east coast waters prior to 1957. A parasite similar to *H. nelsoni* was subsequently seen in *C. gigas* from both California (USA) and Korea.[14, 15] The development of a sensitive and specific DNA probe for *H. nelsoni* has recently enabled researchers to verify the identify of the parasite seen in *C. gigas* as being *H. nelsoni*.[16, 17] This is the most direct evidence to date confirming that the

introduction of an exotic species was responsible for a major epizootic event in a local population.

Viral gill disease in *C. angulata* (France)

The Portuguese oyster, *Crassostrea angulata*, was introduced to the Gironde River in France in 1868 when a boat was forced by a storm to dump its load of (supposedly dead) oysters.[18] By the 1960s, production of *C. angulata* was five times greater than that of the native oyster, *Ostrea edulis*. In March 1966, 900 kg of Pacific oyster, *C. gigas*, seed from Japan was introduced to the Bay of Marennes-Oleron area of France.[19] The following November a new disease, characterized by lesions of the labial gills, was seen in *C. angulata*.[20] From 1970 to 1972, this species developed a second syndrome, which included invasion of connective tissues by blood cells, some having an abnormal size with inclusions, and an increase in the number of brown cells.[21] This epizootic was later demonstrated to be caused by an iridovirus.[22] Massive mortalities occurred, and even though an embargo on further importations was emplaced in the fall of 1967, further declines in stocks led to the decision to import *C. gigas* in commercial quantities beginning in 1970.[19] Adult oysters were imported from British Columbia between 1971 and 1975 and planted directly in the main *C. angulata*-producing bays. From 1971 to 1977, spat from Japan was introduced to reseed oyster grounds. By 1973, *C. angulata* had died out and was replaced in most areas by *C. gigas*. At present, the industry is supplied seed from natural sets occurring in Arcachon Bay and Marennes-Oleron Bay where water temperature is high enough to initiate spawning.[23]

Aber Disease in *O. edulis* (France)

A protozoan, *Martelia refringens*, was associated with mortality of native, European flat oysters, *O. edulis*, which began to die in Aber Wrach in Brittany in 1967 where Pacific oysters, *C. gigas*, were being held.[24] This parasite most seriously affects the intestine and digestive gland tubules and is commonly referred to as "digestive gland disease". Like the gill disease of *C. angulata*, *M. refringens* has little effect on the exotic *C. gigas*, even though it does occur in low prevalences in this species.[25]

Bonamiasis in *O. edulis* (France)

Bonamia ostreae, another protistan parasite, increased to epizootic proportions and further reduced populations of *O. edulis*, beginning in 1979.[26, 27] There is evidence that *B. ostreae* was introduced to France in years prior to 1979 with imports of *O. edulis* seed from a hatchery in California (USA). Broodstock used at this hatchery was offspring of a stock imported from Connecticut, which in turn had been introduced from the Netherlands, beginning in 1949.[28-31] "Microcells" morphologically similar to *B. ostreae* were associated with slow growth and mortality of *O. edulis* in California as early as 1966, while adjacent stocks of *C. gigas* were unaffected.[29] Since becoming established in France, the pathogen has spread throughout Europe. California seed may also have been the source of *B. ostreae* in introduced populations of *O. edulis* in Washington and Maine.[30, 32]

There are three possible explanations for the origin of bonamiasis in *O. edulis*. The first is that oysters were already infected when originally introduced to North America from the Netherlands. This is not likely since epizootics were not seen in Europe until 1979, after the introduction of seed from California. The second possibility is that oysters were infected after introduction to U.S. waters. Even though *B. ostreae* has never been seen in native bivalves from this region, "microcells" resembling *B. ostreae* were seen in *O. edulis* from Milford, Connecticut.[31] The third possibility is that *O. edulis* became infected after being introduced to California waters. As pointed out by Katkansky et al.[29], the microcells seen in *O. edulis* resembled an organism associated with mortalities of Pacific oysters, *C. gigas*, on the west coast of Canada (Denman Island disease) that were documented as early as 1956. In addition, both *C. gigas* and *C. angulata* were present adjacent to infected *O. edulis* in the Katkansky et al.[29] study. The development of a molecular probe specific for the DNA of *B. ostreae* (similar to that developed for *H. nelsoni*) would greatly enhance our ability to not only trace possible routes of disease agent introduction, but also to locate cryptic stages of this parasite, identify alternate hosts, and define its life cycle.

Reducing disease risks

The probability that introductions of exotic species have resulted in massive epizootics in susceptible, local populations, dictates that a means of preventing the transfer of disease agents be determined. Ideally, only pathogen-free individuals would be introduced. Histological examination is routinely used to screen populations for the presence of parasites and pathogens. For example, histological examination of Pacific oyster seed from Japan, Taiwan, and Korea revealed a number of microparasites and potential disease-causing agents.[9] Unfortunately, histological screening of potential seed sources can only reduce, not completely eliminate, the possible introduction of a disease agent. First, known pathogens may not be found if they are in very low prevalence in the sample tested. Every individual in the population being considered for introduction would have to be examined to assure that the prevalence of a particular pathogen is zero. Even then, a pathogen could be missed because it wasn't in the particular section of tissue (5-6 μm) examined. Also, it is possible that the person examining the slides could simply miss seeing the pathogen. Second, even known pathogens may have cryptic stages that are presently unrecognized. Third, the pathogenicity of parasites that are "benign" in the exotic population cannot be estimated for populations

in the local ecosystem. Fourth, there may be general pathological conditions that exist which are not recognized as being associated with a specific agent. These are generally referred to as "idiopathic lesions" or "nonspecific granulomas".[2)]

Another approach to eliminating unwanted disease introduction is to spawn broodstock populations in quarantined (isolated from the natural environment) systems and introduce only the offspring, which would presumably have a reduced parasite load. Even this is not completely effective, as illustrated by the attempt in Canada to introduce the bay scallop, *Argopecten irradians*. Scallops were imported from Connecticut (USA) in 1979 to provide inshore fisherman with a new fishery resource.[33)] To protect native species from potential disease outbreaks, the stock was kept quarantined for three generations before introduction into the Gulf of St. Lawrence. Morrison and Shum[34, 35)] examined some of the originally imported scallops and found chalmydia-like and rickettsial infections, as well as non-specific granulomas. In 1989, after the introduction had occurred, scallops were found to contain a protozoan identified as *Perkinsus karlssoni*.[36)] Re-examination of histological sections from the original scallops introduced in 1979 revealed the same parasite; no similar parasite has been observed in native molluscs from Atlantic Canada.[37)] A similar organism, however, was previously seen in scallops from the United States.[38)] Thus, even rearing an exotic species through several generations in quarantine cannot eliminate the risk of introducing an unwanted parasite, particularly one that is vertically transmitted. Until a means of completely and non-destructively screening each individual to be introduced is determined, there will always be a risk of pathogen transfer with introductions of exotic bivalves.

"Hitchhiker" species

Historically, concern surrounding intentional introductions was for the production of the introduced species, and not for the ecosystem receiving the exotic species. Little care was taken to ensure that only the intended species was introduced. As a result, a number of "hitchhiker" species were introduced along with the intended species. Dundee[39)] reported that there are 204 species of exotic molluscs present in the continental United States. There are an estimated 150-200 species of exotic invertebrates along the Pacific coast of the United States.[1)] Some of these were associated with introductions of marine bivalves. Hitchhiker species may compete with local species for space and resources, prey on local and exotic species, or harbor additional organisms pathogenic to one or more native species. The impact of these additional exotic species on local ecosystems is not generally realized until some time after the original introduction has occurred.

West coast of United States

Shortly after the completion of the transcontinental railroad in 1869, attempts were made to establish the eastern oyster, *C. virginica*, and the hardshell clam, *Mercenaria mercenaria*, in San Francisco Bay.[1, 8)] As reproduction was unsuccessful, repeated introductions of oysters from New England were undertaken (until about 1935). In Washington State, stocks of the native oyster, *Ostreola conchaphila* (=*Ostrea lurida*), were being reduced by overharvesting and disease and the species was slow growing and difficult to culture.[8, 40)] Introductions of *C. virginica* from the eastern seaboard were made between 1900 and 1902.[40)]

Several species of molluscs were introduced to these regions along with *C. virginica*.[8, 41)] The softshell clam, *Mya arenaria*, was abundant in San Francisco Bay, California, after 1874 and in Puget Sound, Washington, by 1884. It is now distributed from Alaska to San Diego, California, because it was able to grow and reproduce at the relatively low temperatures prevalent on the west coast. The oyster drill (a predatory gastropod), *Urosalpinx cinera*, was also introduced. Three species of slipper shells (*Crepidula fornicata*, *C. convexa*, and *C. plana*) were introduced, although only *C. fornicata* became established. The ribbed mussel, *Guekensia* (=*Modiolus*) *demissus*, became common on the warm, intertidal shores of San Francisco Bay. The mud snail, *Nassarius obsoletus*, is now found in warm regions, including San Francisco Bay and Willapa Harbor, where *C. virginica* was introduced.[41)] The transverse ark, *Anadara* (*Arca*) *transversa*, and the whelk, *Busycon canaliculatus*, were also introduced, but did not reproduce successfully. The Atlantic shipworm, *Teredo navalis*, was responsible for the destruction of large numbers of wooden pilings in San Francisco Bay around 1920.[41)]

Given the demise of the native *O. conchaphila* and the lack of reproductive success of *C. virginica*, attention was focused on Pacific oysters, *C. gigas*.[8, 40)] The first attempt to introduce adult *C. gigas* from Japan in 1902 was unsuccessful due to mortalities during shipment. Introductions were sporadic between 1902 and 1919. In 1919, a shipment of large oysters was planted in Samish Bay, Washington; most of these died, but juveniles attached to their shells survived, leading to the practice of importing seed (juvenile) oysters. Large-scale oyster cultivation was occurring by 1928 in the states of California, Oregon, and Washington. Since 1947, over one million cases of seed oysters were introduced.[40)] Most seed is now produced in local hatcheries.

Several hitchhiking marine species were introduced to the western United States along with the introductions of *C. gigas*.[8, 40)] The Japanese littleneck, *Tapes philippinarum* (=*Venerupis japonica*), became established in San Francisco Bay and Humboldt Bay, California, and in Willapa and Grays Harbor and Puget Sound, Washington, where it now supports a considerable industry. The Japanese oyster

drill, *Ceratostoma inornatum* (=*Ocenebra japonica*), was introduced into waters of the Pacific coast of North America with *C. gigas* as early as 1928.[41] The mollusc *Batillaria zonalis*, is now widely established in Puget Sound, Washington and California. The flatworm, *Pseudostylochus ostreophagus*, has killed oyster spat in Puget Sound, Washington. The parasitic copepod, *Mytilicola orientalis*, although generally not lethal, infests the intestinal tracts of mussels and oysters and reduces their condition and value.[40] This parasite has been found in native Olympia oysters (*O. conchaphila*), California mussels (*Mytilus californianus*), *M. crassitesta*, and *M. edulis*.[9] Eelgrass, *Zostera nolti*, has become established in Willapa Bay, Washington, and is filling the bare intertidal space between the native *Z. marina* and *Salicornia*.[1] The phaeophyte, *Sargassum muticum*, is considered a nuisance seaweed, growing in very dense stands in the lower intertidal and subtidal zones.[1, 40]

British Columbia, Canada

The history of oyster introduction to western Canada is similar to that of the west coast of the United States. In British Columbia the native oyster, *O. conchaphila*, was harvested from 1884 to about 1936, when stocks became depleted.[42] *Crassostrea virginica* was thus introduced to Boundary Bay (mainland) in 1906 and to Esquimalt and Ladysmith Harbours (Vancouver Island) in 1903.[41] Although growth of *C. virginica* was excellent, low summer temperatures generally prevented successful reproduction. This neccessitated repeated introductions from New England, which continued until about 1936. *Crassostrea virginica* is now rare on the Pacific coast, although a small population persists in Boundary Bay.[42] Hitchhikers introduced with *C. virginica* include the mud snail, *N. obsoletus*, and the drill, *U. cinera*, which are presently confined to Boundary Bay.[41, 42]

Crassostrea gigas was introduced to Ladysmith Harbour and Fanny Bay in 1912-13 by individual fishermen of Japanese descent.[42] This unauthorized activity continued until 1925 when it became evident that natural breeding was taking place. In 1925, 2000 2-3 yr old oysters from Samish Bay, Washington and 20 cases of seed from Japan were introduced. Regular importation of Japanese seed continued until 1978.[41, 42] Breeding was generally successful in years in which average summer temperatures were above normal. Importations were ceased after Pendrell and Hotham Sounds became consistent sources of seed oysters and as the cost of seed shipment increased and hatchery techniques were developed.[41, 42]

In British Columbia, several exotic species were introduced as the result of oyster (*C. gigas*) culture.[41] The Japanese littleneck, *T. philippinarum*, probably brought in with Japanese seed, was first discovered in Ladysmith Harbour in 1936, and by 1941 had entered the commercial clam harvest.[41] In some areas, *M. arenaria* became the dominant clam, as it filled an intertidal niche not occupied by native clams. This clam has subsequently become widely accepted both ecologically and as food.[41, 42] The Japanese oyster drill, *C. inornatum*, is now common on west coast oyster beds. Another drill, *Purpura clavigera*, was found in Ladysmith Harbour in 1951, but no further specimens have been reported from any region. The most abundant of the introduced gastropods, *Batillaria cumingi*, has a distribution associated with known plantings of Pacific oyster seed; it is abundant in Boundary Bay, Ladysmith, Crofton, Fanny Bay, and Comox.[41] The flatworm, *P. ostreophagus*, has killed oyster spat in Puget Sound (Washington) and Pendrell Sound (B.C.). The parasitic copepod, *M. orientalis*, reduces condition and value of mussels and oysters. The marine wood borer, *Limnoria tripunctata*, now causes serious economic damage. The Japanese phaeophyte, *S. muticum*, considered a nuisance seaweed, has become established in a previously open niche from 0.5 m above low tide to 2 m below low tide.[42]

Western Europe

Crassostrea virginica from the east coast of the United States was introduced to Great Britain annually from before 1900 to 1939 for growth and marketing, as natural reproduction did not occur in the relatively cool waters.[8] Several hitchhiking species were introduced along with *C. virginica*.[1, 8] The slipper shell, *C. fornicata*, which settles on oysters and competes for food, was found in 1893, and is now distributed from Sweden to France. The predatory gastropod, *U. cinera*, was found in 1920 and *Clymenella torquata* was established by 1949. Other species probably introduced with *C. virginica* include the bivalves *Petricola pholadiformis*, *M. arenaria*, and the mud crab, *Rhithropanopeus harrisi*, and the ostracod *Sarsiella zostericola*; all now have a wide distribution in northern Europe.[1, 8]

The large-scale introduction of the Pacific oyster, *C. gigas*, to France in response to disease mortalities (discussed previously) resulted in several unintended introductions.[19] The parasitic copepod, *M. orientalis*, has contributed to periodic, localized mortalities in the Bassin d'Arcachon.[23] In Brittany, an annelid, *Hydroides enzoensis*, a bivalve, *Anomia chinensis*, a cnidarian, *Aiptasia pulchella*, and two species of barnacles, *Balanus amphitrite* and *B. albicostatus*, were also introduced with Japanese spat collectors.[43] The algal species *Undaria pinnatifida* and *Laminaria japonica*, found in the Thou lagoon on the Mediterranean coast, were associated with introductions of *C. gigas*.[19]

Reducing hitchhikers

Early introductions were made with little consideration for hitchhiking organisms; seed was packed directly from the originating water, complete with attached organisms. Reference is made in Andrews[8] to the finding in 1930 of 22 species of marine molluscs in 20 boxes of Japanese seed.

By the time inspection and washing of seed to reduce fouling organisms was undertaken, many species capable of becoming established in the new ecosystem had already gained a foothold. Thus in many cases, establishment of the intended species failed, while the hitchhiking species succeeded, often to the detriment of the local ecosystem. Establishment of hitchhikers has occurred primarily in situations where there is an available ecological niche and where the new ecosystem provides the proper conditions for successful breeding. Even if these conditions are not present every year, repeated introductions may eventually provide hitchhiking exotics with an opportunity to become established.[41]

There are several approaches that might be taken to reduce the introduction of hitchhiker species and their negative impacts on local ecosystems. The first is by more careful culling of material prior to the introduction. An example is provided by the introduction of European oysters, *O. edulis*, from the United Kingdom to eastern Canada between 1957 and 1959, in which each oyster was individually scrubbed clean of fouling organisms.[44] This is labor intensive and does not ensure elimination of all hitchhikers, particularly larval or juvenile stages of marine invertebrates. Another means of reducing hitchhikers is through some sort of disinfection. Medcof [44] immersed oysters in seawater solutions of Lindane in an attempt to kill barnacles. Oysters might also be soaked in a weak bleach solution, hypersaline solution, or freshwater.[23] Unfortunately, the effectiveness of any of these treatments will vary depending on the tolerance of the hitchhikers present relative to that of the intended species. The most effective means of reducing hitchhikers would be accomplished through quarantined spawning of introduced broodstock and then introducing only the offspring. Even though this approach did not totally eliminate the transfer of parasites in the case of the introduction of *A. irradians* to Canada (as discussed previously), it did effectively eliminate hitchhikers.

Direct Competition

The intentional introduction of an exotic species may result in direct biological competition with one or more native species. Exotics may out-compete native species for food and space, essentially replacing the native species in its ecological niche. Competition may be especially intense if the introduced species is capable of faster growth and greater reproductive output than a related native species. If the introduced species can interbreed with a local species, hybridization and a loss of genetic variability might also occur with unknown results.[45] Introduced species may also gain an advantage over local species if they have greater resistance to pathogens (as previously discussed). Through either mechanism, the introduction of an exotic species may ultimately reduce biological diversity in the local ecosystem.[3]

United States

As described previously, the Pacific oyster, *C. gigas*, was introduced to Washington State to augment production of the native oyster, *O. conchaphila*. In Willapa Bay in the 1950s and 1960s, good sets of *O. conchaphila* occurred on Pacific oyster shells; however, at a size of about 1-2 cm they died.[46] Possible reasons for this occurrence are that feces or metabolites from *C. gigas* were affecting survivorship of the native oysters, or that *C. gigas* had essentially outcompeted native oysters for food.[46] Today there are only a few small reefs of native oysters in Puget Sound.

France

The introduction of *C. gigas* to France (discussed previously) has resulted in the virtual elimination of *C. angulata*, particularly in Arcachon Bay and the Bay of Marennes-Oleron, and a reduction in the range of the native oyster, *O. edulis*.[18] In this case, however, the predominance of *C. gigas* was aided by the susceptibility of the other oyster species to diseases.

Australia

As early as 1939, the Australian government was considering options for increasing oyster production in that country. The mud or Port Lincoln oyster, *Ostrea angasi*, once common along the southern coast of Australia, was overfished throughout the 19th century.[47] Attempts to extend the natural range of the Sydney rock oyster, *Saccostrea commercialis*, from the eastern coast to South Australia, Western Australia, and Tasmania had not succeeded. It was decided therefore, to undertake the introduction of the Pacific oyster, *C. gigas*.[47]

In 1947, 55 cases (15,000 oysters/case) of *C. gigas* seed were brought to two sites for trial introductions: Oyster Harbour, Western Australia and Pittwater, Tasmania.[47] Oysters at Oyster Harbour died within a year, but oysters at Pittwater survived, grew, and spawned. Subsequent introductions were made at Pittwater in 1948, 1951, and 1952. Oysters from Pittwater were transferred to Port Sorell in September 1953 and to Mallacoota Inlet in Victoria in 1955, where spatfall occurred in adjacent regions.[48]

New South Wales refused introduction of *C. gigas* stock for fear of competition with the native oyster, *S. commercialis*. In spite of precautions, *C. gigas* did move to New South Wales, probably the result of natural breeding and larval transport from other areas, on the hulls of Tasmanian scallop boats, or in shipments of native spat contaminated with *C. gigas* spat.[42, 49] Five Pacific oysters were found at Pambula in 1967 but nothing further was noticed until 1973 when they appeared in several estuaries.[49] In response, the Fisheries Department declared *C. gigas* to be a "noxious fish" and enlisted local oyster farmers

to destroy all live specimens. In spite of this, large numbers of *C. gigas* were found on commercial leases of *S. commercialis* in Port Stephens in 1985, possibly the result of an intentional introduction. As Port Stephens is the primary *S. commercialis* seed-producing region, *C. gigas* now represents a direct competitor for substrate and resources in New South Wales.[49]

New Zealand

Crassostrea gigas was accidentally introduced to New Zealand via the hulls of ships, as larvae in ballast water, or via larval transport from Tasmania.[42, 50] Six individuals were found in 1971, and by 1972, *C. gigas* had spread to several areas. *Crassostrea gigas* was effectively spread to all oyster-growing areas on spat collectors intended for the native rock oyster, *Saccostrea glomerata*. From 1975 onward, *C. gigas* began to dominate and farmers found it impossible to grow the two species together or separately. Since 1977, *C. gigas* has become the dominant oyster species.[50]

It is clear that *C. gigas* has directly outcompeted the native oyster species in New Zealand. Reproduction of *C. gigas* occurs at the same time as that of *S. glomerata*, so that both species compete for settlement space.[50] In 1972, the ratio of rock oysters to Pacific oysters on a spat collector was 1000 to 1; by 1977 it was 1: 1; in 1978 it was 1: 4 in favor of *C. gigas*. Pacific oysters grow to market size in 15-18 months, compared to the 36 months required for *S. glomerata*. Oversettlement of *C. gigas* spat smothers the smaller *S. glomerata*. As a result of its greater fecundity and faster growth, *C. gigas* superseded *S. glomerata* within a decade of its introduction.[50]

There is also evidence that some hybridization of *C. gigas* and *S. glomerata* has occurred in nature. Even though attempts to cross the two species was not experimentally successful, two rare alleles have been found in *C. gigas* from New Zealand which previously have only been recorded in *S. glomerata*.[50]

Reducing competition

Means of reducing competition from hitchhiking species has already been discussed. The following pertains only to reducing competition with native species that comes from intentionally introduced species. In the previous examples of direct competition, the exotic species occupied the same ecological niche as the native species and due to more rapid growth and greater fecundity, successfully outcompeted the native species for substrate and energy. In some cases (New South Wales and New Zealand), an exotic became established because of an introduction to an adjacent area.

To avoid or minimize competition, there are several approaches that could be taken. First, the exotic species should be chosen so that its ecological niche does not overlap with an existing local species. Second, there should not be areas nearby that might become colonized by the exotic species and negatively impacted. Third, competition can be minimized by introducing non-reproducing individuals, even if there is niche overlap. This can be accomplished by choosing a species that can grow but cannot reproduce successfully in the local ecosystem (i.e, water temperatures are too low). A safer approach would be to use sterile (triploid) individuals.

POSITIVE IMPACTS OF INTRODUCTIONS ON LOCAL ECOSYSTEMS

There are no documented cases of intentional introductions having a positive impact on a coastal ecosystem. Introductions have generally been considered positive if they resulted in economic improvement with no appreciable negative impact on the local ecosystem (e.g., the introduction of *C. gigas* to British Columbia and to the United Kingdom).[42, 51] The argument for making an introduction on ecological grounds is discussed in the next section.

PROPOSED INTRODUCTION OF *C. GIGAS* TO THE EAST COAST OF NORTH AMERICA

Background

The Atlantic coast of North America is the only region in the northern hemisphere in which *C. gigas* is not cultured. This is because until fairly recently, stocks of the native oyster, *C. virginica*, have been plentiful; thus there has been no economic incentive (as in France, the Pacific Northwest, and Australia) to introduce another species. Chesapeake Bay was the leading oyster producing region in the United States from 1885 to 1960.[52] Beginning around 1960, however, the effects of many years of habitat destruction and overharvesting the natural oyster resource of the mid-Atlantic region (Delaware and Chesapeake Bays) of the east coast were exacerbated by two pathogenic parasites, *H. nelsoni* and *P. marinus*, resulting in further reductions in annual landings.[10, 52-54] In Virginia, average annual oyster production exceeded 1.0 million bushels prior to 1950; by 1995 this had decreased to $<$4,000 bushels (Figure 1). Both pathogens have become widely distributed in both bays, although small refuge populations in low salinity areas remain relatively disease free. Since the sudden appearance of *H. nelsoni* in Delaware Bay in 1957, some natural resistance to the pathogen has developed.[10] A similar tolerance to *P. marinus* has not developed in natural populations, and oyster strains selectively bred for resistance to *H. nelsoni* are susceptible to *P. marinus*.[55]

Mann et al.[56] argue that *C. gigas* should be introduced to Chesapeake Bay not solely for economic reasons, but for the positive impact the species would have on the local ecosystem. As pointed out by Gottleib and Schweighofer,[57]

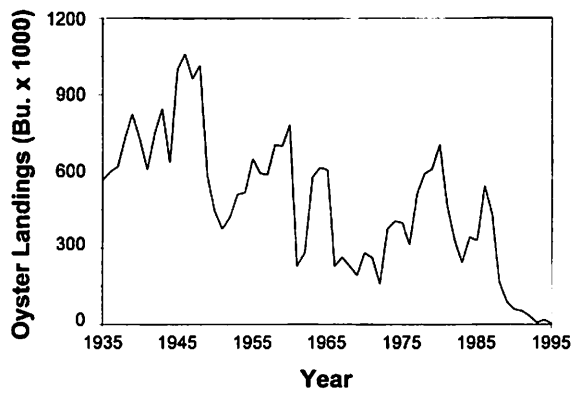

Figure 1. Landings of market oysters (bushels) from public oyster beds in Virginia, 1935-1995. One bushel $\approx$ 0.05 m^3. Source: Hargis and Haven[52] and the Virginia Marine Resources Commission.

bivalve molluscs are esse ntial components of healthy estuaries throughout the world. Oyster reefs have historically been important biological and ecological structures that supported extensive communities, which in turn support other valuable fisheries. Centuries of mining oysters from the bottom of Chesapeake Bay, combined with recent disease mortality, have greatly reduced the area of living reef in the bay. Rothchild et al.[54] estimated that total oyster habitat in the Maryland portion of Chesapeake Bay is 50% or less of what it was a century ago, and that the biomass per unit of remaining habitat is about 1% of that existing in 1900. Once oysters die or are removed, reefs rapidly become silted over and lost as substrate for other organisms. A second ecological consideration is the role that oysters play as consumers of primary production. Based on standing stock estimates, Newell[58] estimated that the oyster standing stock prior to 1870 was capable of filtering the entire volume of the bay in 3.3 days; in contrast, it takes 325 days today. The 100 fold decrease in oyster standing stock since 1870 has resulted in a general eutrophication of bay waters accompanied by seasonal periods of hypoxia.[56,57] An increased oyster biomass in Chesapeake Bay would result in increased water column filtration, increased sedimentation and organic production in the sediments, and increased nutrient cycling.[56-58] Indeed, several accidental introductions of bivalves appear to have resulted in improved water quality in several estuaries.[57]

The idea of introducing *C. gigas* to Chesapeake Bay (Virginia) has gained momentum in recent years in relation to the declining harvests of *C. virginica*. Proponents of the introduction base their arguments on the seemingly irreversible demise of the native *C. virginica* and the related economic and ecological ramifications. Opponents of the introduction fear that unknown pathogens or hitchhikers that might affect other species in the bay would accompany *C. gigas*.[59] There is fear that *C. gigas* will outcompete *C. virginica* by growing faster and reproducing more successfully. This would possibly lead to spat crowding or increased biodeposition that would smother beds of *C. virginica*. There is also a concern that *C. gigas* might hybridize with *C. virginica*. Other states on the east coast oppose the introduction for fear that once introduced to Chesapeake Bay, it will be difficult to keep *C. gigas* out of areas to the north and south where *C. virginica* is still viable. This proposed introduction has provided an opportunity for careful evaluation of all these concerns, utilizing what has been learned from previous introductions, augmented by results of recent research.

Approach

The potential for introduction of pathogens to Chesapeake Bay along with *C. gigas* is addressed by Mann et al.,[56] who conclude that the introduction of all known potential disease agents except viruses, bacteria, and the ovarian parasite, *Marteilioides chungmuensis*, can be avoided by introducing only progeny of broodstock spawned in quarantine. Both the viral and bacterial pathogens tend to be universally distributed and can be limited with careful husbandry. *Marteilioides chungmuensis* is not known to cause mortality in *C. gigas*, but its impact on *C. virginica* or any other native species in Chesapeake Bay cannot be ignored. The possibility exists that presently unknown or unrecognized pathogens might also be introduced (e.g., the introduction of *P. karlssoni* with *A. irradians* to Canada). Thus although there is a risk of disease outbreak associated with the introduction of *C. gigas* to Chesapeake Bay, it is minor. Also, if only progeny of broodstock spawned in quarantine were utilized, virtually all risk of introducing hitchhiking organisms would be eliminated.

Most of the concerns regarding the introduction of *C. gigas* to Chesapeake Bay revolve around biological competition with the native *C. virginica*. In no previous introduction of *C. gigas*, however, has *C. virginica* been the native species, so prediction of potential species interaction is impossible. It is also difficult to predict the survival, reproductive success, recruitment, and ultimate geographic distribution of *C. gigas* in Chesapeake Bay merely by comparing prevailing environmental conditions with those in locations where *C. gigas* has become established. To address these concerns, a series of experiments (limited by the constraints of following the International Council for the Exploration of the Sea (ICES) protocol to prevent unwanted introductions) were undertaken to determine the ability of *C. gigas* to survive, grow, and reproduce relative to *C. virginica*, in the environment of Chesapeake Bay. A continual supply of bay water (York River, Virginia) was pumped to flumes holding oysters; water was discharged to a holding basin on land where it percolated into the sandy bottom.

Results

Disease tolerance

The first question to be answered was whether *C. gigas* could survive in Chesapeake Bay. Even though the environmental preferences of *C. gigas* for temperature and salinity are generally met in Chesapeake Bay, this alone is not enough to predict the level of tolerance to the pathogens *H. nelsoni* and *P. marinus* prevalent in the bay.[56] Meyers et al.[60] challenged diploid and triploid *C. gigas* and *C. virginica* with *P. marinus* and found that after 83 days, prevalence of infection (using the thioglycollate technique) was lower in *C. gigas* (40%) than in *C. virginica* (100%); *C. gigas* exhibited only "light" infections while *C. virginica* had "moderate" and "heavy" infections; cumulative mortality after 150 days was 100% in *C. virginica* and <35% in *C. gigas*, with no difference between diploid and triploid groups. Thus initial indications were that although *C. gigas* is susceptible to infection by *P. marinus*, it is considerably more resistant to disease compared to *C. virginica*.

Because *H. nelsoni* cannot be transmitted from oyster to oyster, similar experiments to examine the tolerance of *C. gigas* to this pathogen in quarantine conditions have been problematic. Some exposure to *H. nelsoni* undoubtedly occurred in the flume experiments (described below) , but no infections were observed. Ultimate challenge with this parasite can only be accomplished *in situ*, which by definition is an introduction. Nonetheless, *C. gigas* individually certified as being triploid (to avoid possible spawning) were exposed to *H. nelsoni* in the waters of Chesapeake Bay; the *C. virginica* controls became highly infected and suffered high mortality while no infections were seen in *C. gigas* and mortality was low (E. Burreson: Virginia Institute of Marine Science, Gloucester Point, Virginia, pers. commun.). Based on the observations to date, it appears that *C. gigas* is not susceptible to infection by *H. nelsoni*.

Growth and Mortality

To examine the ability of *C. gigas* to survive and grow in Chesapeake Bay relative to *C. virginica*, Barber and Mann[61] compared growth, mortality, and disease prevalence and intensity (using the thioglycollate technique) among cohorts of *C. gigas* and *C. virginica* under challenge from *P. marinus*. Both cohorts were produced in April 1991. In November 1992, at age 19 months, mean shell height (55 mm) of *C. gigas* was significantly greater ($P \leq 0.05$) than that (41 mm) of *C. virginica* (Figure 2). Over the same time period, cumulative mortality of *C. gigas* was 70% compared to 59% for *C. virginica* (Figure 3), in spite of the fact that prevalence and intensity of infections of *P. marinus* were greater in *C. virginica* (Figure 4). For *C. virginica*, the period of greatest mortality corresponded with the development of advanced *P. marinus* infections. In contrast, greatest mortality of *C. gigas* occurred when salinity was below 20 ppt (before exposure to *P. marinus*). This study corroborated the previous finding that *C. gigas* is highly tolerant of *P. marinus* but also suggested that non-disease mortality in this species may be high in low salinity regions of Chesapeake Bay. This study also demonstrated that under the experimental conditions, *C. gigas* grows faster than *C. virginica*.

Reproduction

To further determine the potential for *C. gigas* to become established in Chesapeake Bay and possibly outcompeting the native *C. virginica*, Barber[62] examined gametogenesis in groups of both species held in separate flumes receiving water from the York River, Virginia (conditions identical to the previous study). Mean shell height of *C. virginica* was 68.3 mm and that of *C. gigas* was

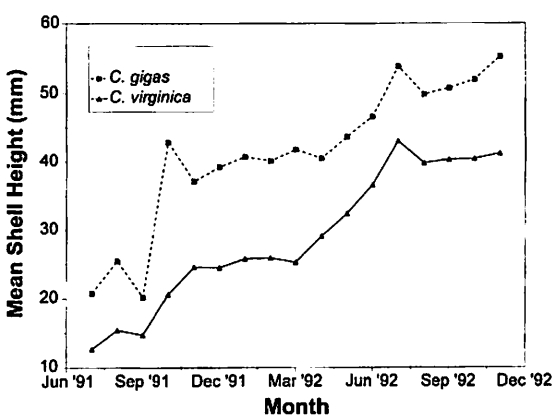

Figure 2. Comparison of growth rate (mean shell height, mm) between *C. virginica* and *C. gigas* held in quarantined flumes in lower Chesapeake Bay from July 1991 to November 1992 (n=100 per species). Source: Barber and Mann.[61]

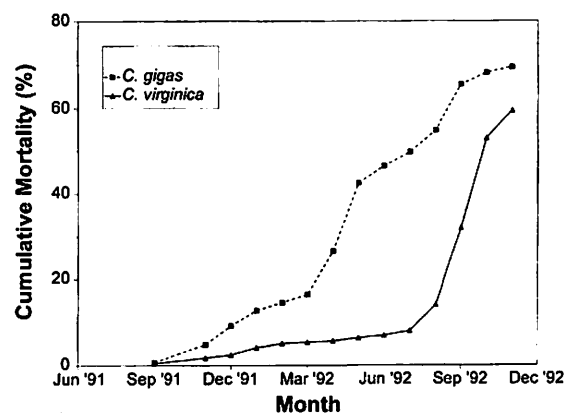

Figure 3. Comparison of cumulative mortality (%) between *C. virginica* and *C. gigas* held in quarantined flumes in lower Chesapeake Bay from July 1991 to November 1992. Source: Barber and Mann.[61]

73.6 mm at the beginning of the study. From March to October 1992, histological examination (n=15 / species / month) revealed that *C. gigas* underwent a complete, synchronous cycle of gamete development, maturation, and spawning (June-July) while *C. virginica* exhibited a general lack of development, maturation, and spawning. In addition, image analysis revealed that *C. gigas* had a significantly greater ($P \leq 0.001$) mean gonadal area index (GAI) than *C. virginica* (Figure 5). Over the same time period, the combined prevalence of *H. nelsoni* and *P. marinus* reached 86.7% (many advanced cases) in *C. virginica* while neither parasite was detected (histological examination) in *C. gigas*. It is likely that the reduced gametogenic capacity observed in *C. virginica* was disease related. Thus the tolerance of *C. gigas* to both parasites prevalent in Chesapeake Bay give it a distinct reproductive advantage over *C. virginica*. The probability exits that *C. gigas* would outcompete *C. virginica*, especially in disease endemic areas (which also happen to be the areas of higher salinity).

To reduce the reproductive capacity of *C. gigas*, and thus eliminate its direct competition with *C. virginica*, triploid oysters, which are effectively sterile, could be utilized.[63] The most common method for producing triploids (treatment with cytochalasin B), however, rarely results in production of 100% triploidy. This means that at least some of the treated embryos in any batch could develop into reproductively competent individuals. The recent breakthrough in producing tetraploid oysters makes it possible to ensure 100% triploid production by mating tetraploids (2n gametes) with diploids (1n gametes).[64] This capability will be an important tool for minimizing impacts of introductions in areas like Chesapeake Bay, where there is concern about competition with native species.

Hybridization

Early reports that *C. gigas* and *C. virginica* produced viable hybrids were based on successful spawning and fertilization of gametes and survival of larvae, rather than on genetic confirmation. Allen et al.[65] demonstrated that true hybrids of *C. virginica* and *C. gigas* can be produced, but that they grow little and die within 10 days. Thus there is no potential for genetic competition between the two species.

Summary

Using a careful experimental approach, much has been learned regarding the potential outcome of an introduction of *C. gigas* into Chesapeake Bay. Using ICES protocol, the likelihood of introducing hitchhiking organisms is minimal. The chance of introducing pathogenic organisms is reasonably low, although the possibility still exists that presently unknown pathogens may exist or those thought to be harmless to oysters may turn out to be lethal to other organisms in the region. The question of possible competition with the native *C. virginica* can be addressed with some certainty. *Crassostrea gigas* should thrive in areas of the bay where prevailing salinity is above 20 ppt. This encompasses the areas where *C. virginica* has been most profoundly affected by *H. nelsoni* and *P. marinus*. In these areas, *C. gigas* will have a distinct growth and reproductive advantage over *C. virginica*. The rate of larval survival and dispersal and ultimate geographic distribution within Chesapeake Bay and beyond, remain unanswered. All questions of competition could be resolved, however, if only non-reproductive individuals (triploid) were utilized. This would necessitate the utilization of only hatchery-produced seed, which might not be cost effective. On the other hand, if enough living oysters were placed into the bay, eutrophication and hypoxia might be reduced as a result of increased grazing of phytoplankton.

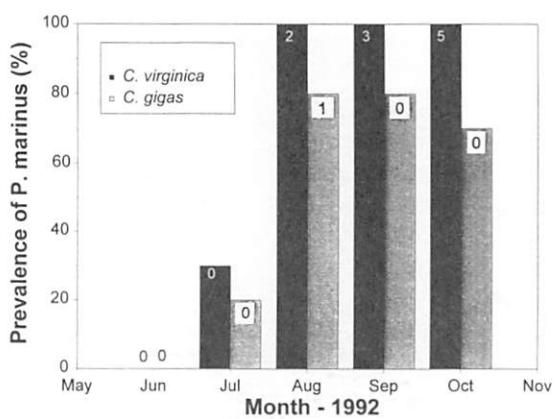

Figure 4. Prevalence (%) of *P. marinus* infection in oysters, *C. virginica* and *C. gigas* held in quarantined flumes in lower Chesapeake Bay (n=10 per species). Initial exposure to the pathogen was in June 1992. Numbers indicate systemic (advanced) infections. Source: Barber and Mann.[61]

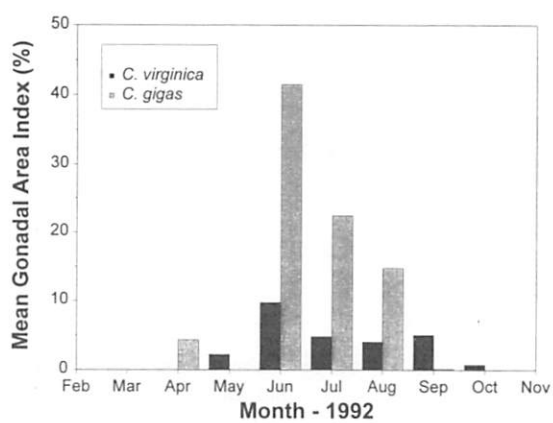

Figure 5. Mean Gonadal Area Index (%) of oysters, *C. virginica* and *C. gigas* held in quarantined flumes in lower Chesapeake Bay, from March to October 1992. Source: Barber.[62]

CONCLUSIONS

Introductions of exotic marine bivalves have generally been undertaken for economic reasons with little regard for impacts on local ecosystems. Three primary negative impacts have occurred.

First, there have been several instances of disease epizootics in a native population after an introduction. It is likely that the exotic species harbored known or unknown pathogens which, when introduced to a susceptible local population, caused disease mortality without itself being affected. It should be possible to eliminate the introduction of all but vertically transmitted parasites by spawning broodstock in quarantine and introducing only progeny (following ICES protocol).

Second, hitchhiking species (non-intentionally introduced) have become established in local environments with varying consequences. Some of these are now prevalent in the new location, while the species intended to be introduced has perished. Hitchhikers have become established where there is an available ecological niche and where the new ecosystem provides the proper condition for breeding. Virtually all hitchhikers can be eliminated by spawning broodstock in quarantine and introducing only offspring (following ICES protocol).

Third, the introduced species may become established (locally or remotely) and because of superior growth rates or reproductive capacity, outcompete one or more local species. This has occurred in instances where the ecological niche of the exotic species is already occupied by a native species (which may be stressed by overharvesting or disease). To reduce or avoid these impacts, only open niches should be targeted for introductions. The competitive ability of an exotic species would be reduced if it was introduced into an environment that was not conducive for reproduction (i.e., temperature or salinity too low). Competition resulting from reproductive activity could be almost eliminated if only sterile (e.g., triploid) individuals made up the introduced population.

The proposed introduction of *C. gigas* to Chesapeake Bay on the east coast of the United States has resulted in much concern and speculation about what the outcome might be. Some of this is based on the negative impacts previous introductions around the world have had on local ecosystems. On the other hand, *C. gigas* would provide a much needed filter feeder to the bay ecosystem. Research has indicated that in certain areas of Chesapeake Bay, *C. gigas* probably would out-compete *C. virginica* for substrate and energy. The use of triploid oysters, however, makes these concerns less viable. Nonetheless, there remains a pervasive fear of the unknown. This introduction will not occur until there is a concensus that the potential environmental risks associated with the introduction are outweighed by the potential ecological and economic gain.

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Bull. Natl. Res. Inst. Aquacult., Suppl. 3 : 155－156 (1997)

INTRODUCTION OF EXOTIC SPECIES AND BIOHAZARD

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The release of artificial fish seedlings into natural waters to sustain the natural bioresources has been traditionally adopted by Japanese fishermen. Also, exotic fishes were actively introduced into freshwater in Japan, resulting in the establishment of new commercial fishes, such as rainbow trout (*Oncorhynchus mykiss*). However, some of them often have become pest species in the Japanese natural ecosystem, bringing such biological hazards as ecological disturbance and genetic pollution.

The mechanisms in the Japanese indigenous fishes were reduced by the introduction of exotic fishes, exemplified by the two cases below.

Bass problem

Largemouth bass (*Micropterus salmoides salmoides*), or black buss the Japanese common name (Figure 1), was initially introduced from the USA to Lake Ashinoko near Mt. Fuji in 1929. The bass had strictly been confined to the lake until the 1960s when lure fishing was introduced from the USA. The bass easily spread into all freshwater in Japan by personal transplantation, because there had been no such perch-like competitor. Since then, the composition of Japanese indigenous ichthyofauna has been changed, with exposure to strong feeding pressure by the new predator.

The bass established in freshwaterin Japan, feed on a wide variety of aquatic organisms as has been reported in the USA. For example, the stomach contents of the bass from Lake Ushiku contained 22 items: 10 fishes, 4 crustaceans, 5 insects, 1 anuran, 1 bryozoan (*Pectinatella magnifica*), and many unidentified plants [1]. It is notable that the bass feed well on crustaceans throughout the year, and on fishes from summer to fall. Their feeding habit will reduce some endemic species not only through the prey-predator relationship but also through the change of the original food-web to a simple and unstable system. This often occurs in the introduction of an exotic fisheater into lakes without fisheaters, i.e. *Cichla* to Lake Gatun in Panama and *Lates* to Lake Victoria in Africa [2].

Figure 1. Largemouth bass, or black bass, the Japanese common name *Micropterus salmoides salmoides* introduced from the USA to freshwaters in Japan.

Genetic pollution

The continental rosy bitterling (*Rhodeus ocellatus ocellatus*, Figure 2A) contaminated in the seedlings of the Chinese major carps (*Ctenopharyngodon idellus, Mylopharyngodon piceus, Hypophthalmichthys moltrix* and *Aristichthys nobilis*) from the Yangtze-Kiang, Changjiang, during the world War II [3]. The Japanese endemic subspecies (*R. ocellatus kurumeus*, Figure 2B) originally inhabited the western part of Japan where the continental subspecies soon invaded. Both subspecies are well distinguished by the coloration along the anterior margin of the ventral fins; the continental subspecies has a remakable white band while the Japanese subspecies has no such band at all [4,5]. They easily hybrized to produce a reproductive F_1 with a white band along the anterior margin of the ventral fins. The appearance of the white band follows the Mendelian rule as a superior character [6]. In the populations of the Japanese subspecies, if genetically introgressed by the continental subspecies, bitterling produce a white band soon.

The genetic pollution through the hybridization with the continental rosy bitterling results in large scale reduction in the Japanese pure form, which are now confined to a few ponds in Osaka and the Chikugo River in Fukuoka. Nagata *et al.*[7] confirmed it in terms of electrophoresis, demonstrating two loci, *Ldh*-2 and *Pgdh*, as diagnostic markers for the identification of both subspecies. On the basis of the karyotypic analysis, Kawamura and Hosoya[8] elucidated the complete displacement of the Japanese rosy bitterling with the continent subspecies in

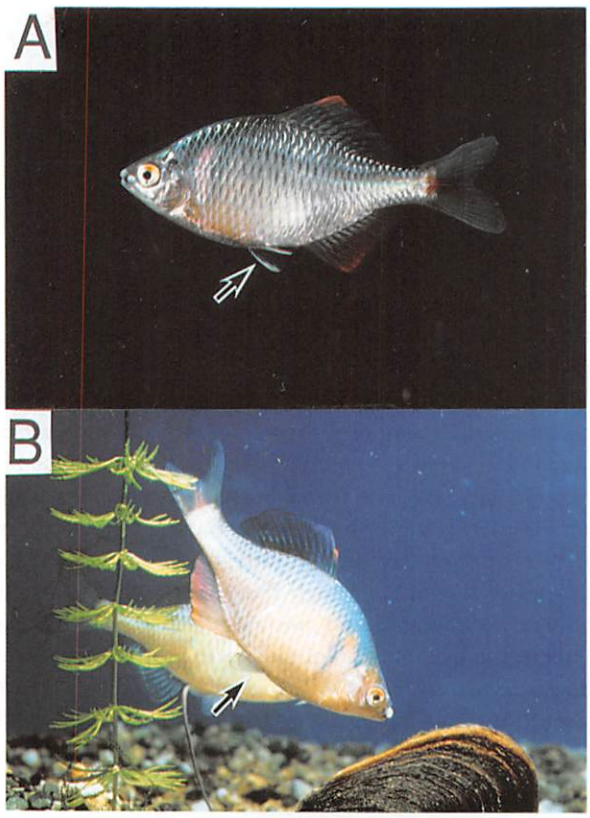

Figure 2. Two subspecies of the rosy bitterlings. A. The continental rosy bitterling, *Rhodeus ocellatus occelatus*. B. The Japanese rosy bitterling, *R. ocellatus kurumeus* (courtesy of Lake Biwa Museum). Note the difference of the coloration along the anterior margin of ventral fins in males by an arrow.

the genetically polluted population.

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RISK TAKING RELEASE FROM NONNATIVE STOCKS OF AYU

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ABSTRACT

Ayu (*Plecoglossus altivelis*), an osmeroid fish, is widely distributed over the Japanese Archipelago and is commercially important for freshwater fisheries. Recent decline in the abundance of the amphidromous populations in rivers has promoted the introduction of nonnative stocks with landlocked populations in Lake Biwa. Difference in competitive ability for foods between native amphidromous and introduced landlocked populations may cause reduced fecundity for the native population. Furthermore, putative spawning intrusion by introduced fish may disturb the genetic diversity of the native population and bring about maladaptive characteristics.

INTRODUCTION

Maintenance of healthy populations is often inconsistent with human consumption and demand for those resources. Overexploitation and habitat alterations result in reduction and fragmentation of local populations. For the sake of stock enhancement, transplantation is conducted with introduced stocks which are usually nonnative. Many local populations of ayu (*Plecoglossus altivelis*), one of the most important fish for freshwater fisheries, reduce their habitat in rivers mainly due to diverting the course of migration. Recent decline in their abundance has promoted transplantation with nonnative fish. Generally, native population is at a risk following the introduction of nonnative stocks through competitive exclusion or introgression.[1,2] For the conservation strategy, the assessment of population-specific traits and the following evaluation of impact of introduced fish on native population are needed.

Ayu, ranging over Japan, migrates between the sea and river amphidromously.[3] Just after fall hatching in the lower reaches, larvae drift downstream to the sea where they feed on zooplankton until the spring upstream migration.[4] During their summer growing season, feeding territories are established in the middle reaches. Territorial individuals are nice game fish because of their fighting. In fall, they become sexually mature and migrate downstream to the lower river reaches to spawn and then die. Migratory behavior like that of amphidromous ayu is also observed in a landlocked population in Lake Biwa, where ayu migrates between the lake and its inlet stream -s.[5] Fish from the Lake Biwa population is supplied as a major stock for the transplantation.

The extent of disturbance of native population by nonnative fish potentially depends on the extent to which individuals are adapted to the local environments, and on the amount of ecological and genetic differentiation between the native and nonnative fish.[6] In the first half of this paper, I overviewed the ecological differences between amphidromous and landlocked populations of ayu. Persistent problems encountered in the management of native populations include recognizing genetically and demographically discrete populations and the functional units of management. Thus, the second half of this paper describes the present genetic status of local populations of ayu.

ECOLOGICAL TRAITS

During the summer growing season, the young feed on algae attached on stones and rocks. Competition for the attached algae is intense, and fish of higher social rank establish territory to monopolize algae. Territorial individuals defend algae against those without territories, and consequently variation in competitive ability among individuals produces a wide range of body size within a population.[7,8] Pairwise experiments designed to compete for algae between amphidromous and landlocked fish in a closed system showed that landlocked individuals had a competitive advantage over amphidromous ones,[9] indicating that landlocked fish grow larger than amphidromous ones through competition for foods in rivers where they are introduced. Ayu become sexually mature regardless of body size within an annual life schedule. Fecundity depends on body size at maturation, and the introduction

of landlocked fish therefore may cause reduction in the potential fecundity of the native amphidromous fish at population level.

Landlocked fish spawn larger number of smaller sized eggs than amphidromous fish,[10-12] which is adaptive to the Lake Biwa environments. However, the low survival of landlocked larvae in the seawater due to their smaller sized body[13] especially during higher water temperature periods[14] makes landlocked fish maladaptive to reproduce in the amphidromous environments. Spawning periods of native amphidromous and introduced landlocked fish are separated in the southern part of Japan, but become close with latitude and overlap in the northern part of Japan.[12,15] When amphidromous and landlocked fish share spawning site, they mate indiscriminately.[16] Spawning intrusion of introduced landlocked fish is accompanied by introgression of maladaptive characteristics to the native amphidromous fish through cross-mating, and brings risk to reduce the fitness return of the native fish.

GENETIC STATUS

Twelve local populations of ayu, including 3 mainland populations, 7 island populations, and 2 landlocked populations in Lake Biwa, were surveyed according to their genetic status. The sequence of the first half of the mitochondria DNA (mtDNA) control region, which is the most quickly evolving and thus the most variable segment as a genetic marker, was analyzed for 10 individuals in each population. Amplification of DNA was conducted by PCR according to standard protocols.[17] Amplified double-stranded DNA was sequenced on an automated DNA sequencer, and 340 base pair sequences from the beginning of the control region were determined.

Intrapopulational nucleotide diversity based on difference in pairwise sequences varied between populations, relating positively to population size. Populations small in size are probably under strong effect of genetic drift, and thereby easy to lose genetic diversity. Marginal populations such as island populations are considered to be more vulnerable to genetic disturbance. As for interpopulational genetic diversity, net nucleotide diversities showed heterogeneous components within a species, showing that ayu has metapopulational structure. Between mainland amphidromous and landlocked populations, population-specific nucletide substitutions were found on some sites with significant difference in the frequency.

CONCLUSION

Introduction of landlocked fish into the habitat of amphidromous fish causes ecological conflict for food resources and may result in reduced fecundity for the native amphidromous population. Furthermore, where spawning intrusion by introduced fish occurrs, the native population may be damaged by the disturbance of population-specific genetic diversity as well as the introgression of maladaptive characteristics. I propose the best method in stock releasing is that stocks should be derived from the native population, produced in each year by using a number of parents to avoid reduction in genetic diversity.

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ARE CAPTURE FISHERIES FOR PACIFIC SALMON IN A CRISIS OF ABUNDANCE ?

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ABSTRACT

Total world salmon production from capture and farmed fisheries in 1995 was about 1.5 million mt as each fishery reached record production levels. This level represents a continuation of recent trends for increased production in both fisheries and in lower prices paid to fishermen and farmers. Continued increases in farmed salmon production have accentuated evolution of a worldwide, two-tiered market demand for salmon products that has had a major impact on the economic viability of all salmon fisheries and salmon products everywhere. The two-tiered system includes moderate-to-low value products dominated by pink and chum salmon and premier market products represented by all farmed fish (mostly Atlantic and coho salmon), and capture fisheries for sockeye, chinook, and coho salmon. Farmed salmon accounted for about 60% of the volume and 70% of the value in the premier quality tier in 1995. In the moderate-to-low value tier, record production of both pink and chum salmon in 1995 was about half of world salmon production, further depressing prices paid to capture fisheries and creating a backlog of unsold product affecting future fisheries.

Stock enhancement programs for Pacific salmon, wild-hatchery interactions, the need for new markets and development of new user-friendly forms of salmon products and trends in ocean productivity of salmon are discussed.

INTRODUCTION

Although depressed runs and endangered stocks occur in some regions, overall, salmon production around the North Pacific Rim is currently at historic high levels of abundance. Capture fisheries for Pacific salmon in Alaska in 1994 and 1995, for example, reached record levels of 197 million and 218 million fish, respectively. The 1995 Alaska harvest yielded 477 million metric tons (mt) of salmon. Pink salmon (*Oncorhynchus gorbuscha*) comprised 60% of the number of fish caught and 44% of the weight, while sockeye salmon (*O. nerka*) comprised 29% and 36%, respectively, of the numbers and weight of the record 1995 Alaska salmon harvest.[1] Salmon fisheries in Japan also reached record levels in both 1994 and 1995 with catches of 68.9 million and 77.2 million fish, respectively, during those years. The record 1995 harvest in Japan yielded 246 million mt, roughly 75% came from Hokkaido and 25% came from Honshu.[2] Over 90% of the Pacific salmon harvested in Japan in capture fisheries consist of chum salmon (*O. keta*).

The record 1995 landings of Pacific salmon in both Alaska and Japan should have been welcome news to fishermen harvesting salmon. In both countries, however, this was not true due to a continued long-term decline in exvessel prices paid to fishermen for their catches. Record 1995 harvests of salmon in both Alaska and Japan yielded less monetary value to fishermen than catches made in 1994. Capture fisheries for Pacific salmon, in general, are only part of dynamic and highly volatile world salmon market forces that are beyond control of any single country, region, or point source of salmon. The ultimate value of all salmon including those caught in capture fisheries is determined by supply and demand and these dynamic market forces.[3] Roughly one-third of total world salmon production in 1995 originated from farmed salmon and two-thirds from capture fisheries.

CAPTURE FISHERIES VS. FARMED SALMON

Supply and demand of salmon products in world markets is greatly influenced by the continued growth of farmed salmon[4] that reached a new record production level of over 500 thousand mt in 1995.[5] Capture fisheries for salmon from all Pacific Rim countries also reached record levels in 1995 with an estimated harvest of 1,044 thousand mt, bringing total world salmon production to an estimated 1.544 million mt. This level of production represents a 50% increase in world salmon production in the five

years since 1990; an increase that occurred during a period when total world fish production was declining (Figure 1). Increases in the world supply of salmon has created an oversupply that has forced prices downward in both capture and farmed fisheries and a search for new markets and new product forms on uses for salmon.

Salmon farming is practiced in roughly 20 countries centered in Europe but also with significant production from North and South America, and Japan.[4] Norway, the leading producer of farmed salmon likely exceeded 275 thousand mt in 1995 (Figure 2). This was in spite of efforts to slow production through restricted feeding programs to limit growth during certain periods.[6] Chile is the second largest producer of farmed salmon. While farmed salmon production in Norway is mostly from Atlantic salmon (*Salmo salar*) production in Chile has focused on both Atlantic salmon and coho salmon (*O. kisutch*).

Production of anadromous Pacific salmon from capture fisheries for these fishes currently involves four principal countries: Russia, Japan, Canada, and United States, with minor participation by Korea and China. Principal salmon-producing Pacific Rim countries all have well developed hatchery programs designed either to mitigate natural spawning or rearing habitat losses or to supplement natural production for other reasons. Production originating from wild, naturally spawning fish and from enhancement or hatchery programs varies considerably among the producing countries and in regions within countries (Figure 3). In the United States, for example, Alaska is the principal source of salmon caught in capture fisheries and hatchery production there has ranged roughly from 14-30% of salmon harvested in recent years. By contrast hatcheries account for over half of the salmon harvested in

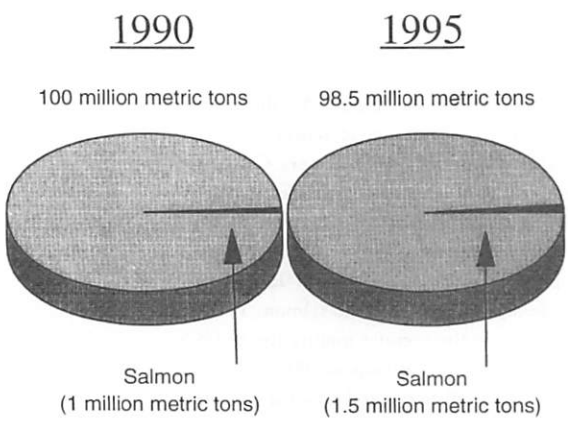

Figure 1. Estimates of total world fish production compared with salmon production in 1990 and 1995.

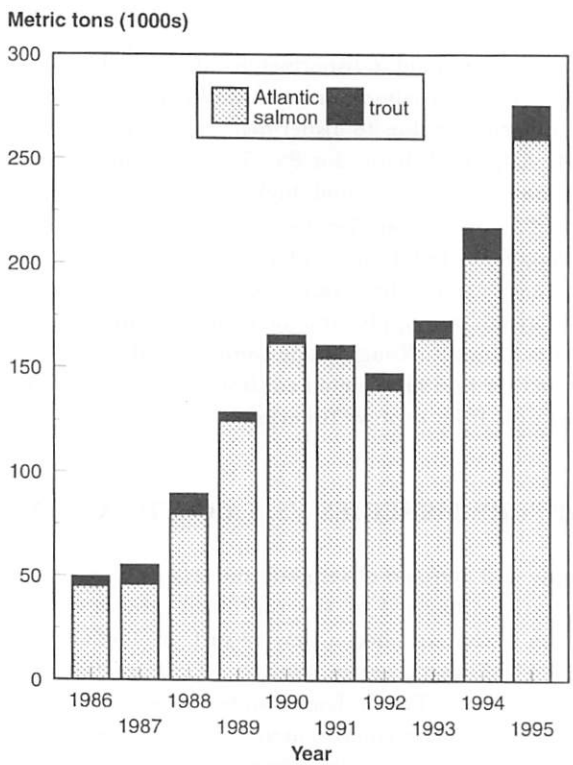

Figure 2. Estimates of Norwegian farmed salmon production 1986-1995. Source:[6]

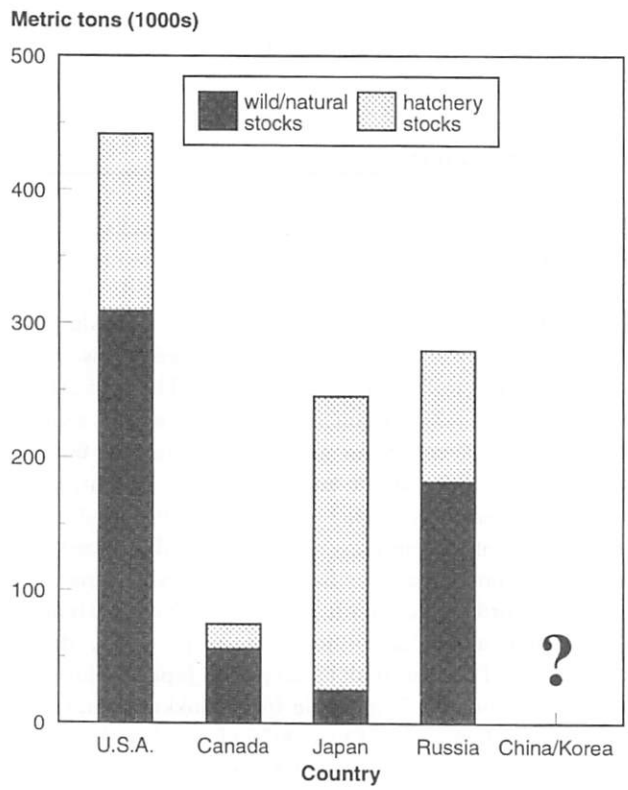

Figure 3. Estimated production of anadromous Pacific salmon landed in capture fisheries in 1995 by country showing proportion originating from natural spawning stocks and hatchery stocks of fish. Total 1995 Pacific salmon caught in capture fisheries was about 1.044 million mt.

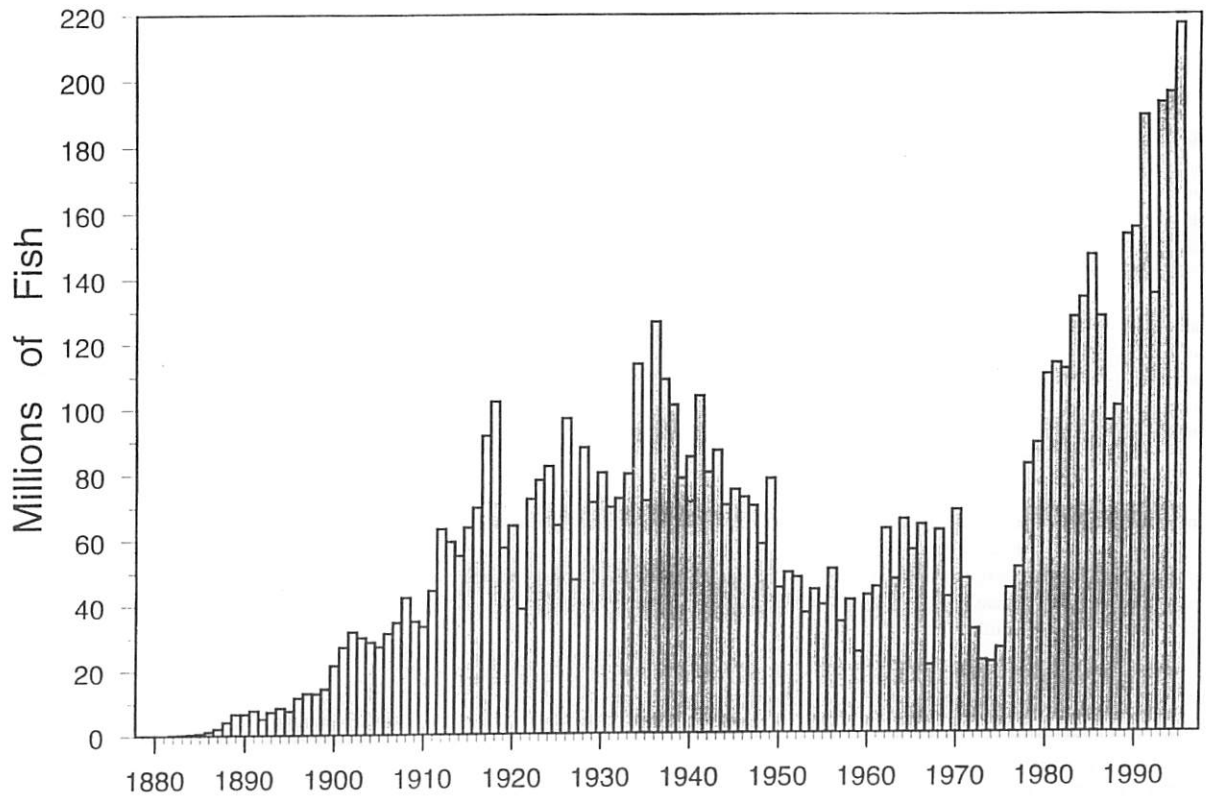

Figure 4. Alaska commercial salmon harvest 1886-1995.

Pacific Northwest states (Washington, Oregon, Idaho, California).[7] Hatchery production comprises most of the salmon caught in capture fisheries in Japan, and in Russia hatcheries in the Sakhalin-Kurile Islands region play a major role in salmon production from that country.

Currently the annual input of juvenile salmon into the North Pacific Ocean, from all sources, has been roughly projected at about 25 billion fry and smolts. The proportion of hatchery and wild salmon in this projection is estimated at about 5.5 billion hatchery juveniles[8] and 20 billion wild juveniles.[9]

SOME ECONOMICS OF CAPTURE FISHERIES FOR SALMON

Simplistic economic models for capture fisheries of Pacific salmon involve long term amortization of capital projects including hatcheries, various harvesting expenses, annual operating cost for management and enhancement efforts, scaled for different production levels plus other variables, and, very importantly, realistic assumptions for survival rates, run strength forecast, and market values for adult salmon produced.[10,11,12] Wide swings in interannual marine survivals, that for the most part are poorly understood, have significant economic consequences. For example, high marine survivals in Alaska salmon that are, at least partially, responsible for record runs in recent years (Figure 4), have been associated with El-nino warming episodes. The effects of these same oceanographic events appear to be reversed in more southerly North American salmon stocks where in Oregon, Washington, and parts of British Columbia marine survivals have generally been low.

Economics of capture fisheries for salmon is a function of many complex issues that vary widely at different times and in different areas. Often viewed only in the context of commercial fish sales for food markets, recreational fisheries and associated activities, also play a major role in the economic viability of capture fisheries in harvesting anadromous salmon. In Iceland, for example, based on the lack of coastal commercial fisheries, the proprietary recreational use of salmon in Islandic rivers is a highly profitable endeavor.[13,14] Sport fishing in Japan, by contrast, is only a marginal factor, yet the Japanese have operated perhaps the most economically viable salmon enhancement program in the world;[15] at least until recent worldwide declines in commercial salmon prices began to adversely affect markets for capture fisheries. Most fisheries in North America, based on a variable mixture of sport and commercial fishery values and in some cases, subsistence fisheries, fall somewhere between the examples of Iceland and Japan.

In both freshwater and marine waters, recreational

Table 1. Annual cost[1] per smolt, 1988 to 1992, at two hatcheries operated by a regional aquaculture association in Southeast Alaska. Values are in U.S. dollars.

Hatchery and Species	Age at Release[2]	1988	1989	1990	1991	1992	Weighted 5-year Average
Neets Bay							
Coho	1+	0.369	0.327	0.259	0.417	0.357	0.323
Chinook	1+	0.485	0.435	0.295	0.472	0.403	0.414
Chinook	0+	-----	-----	-----	0.141	-----	0.120
S. Chum	0+	0.027	0.023	0.019	0.027	0.023	0.024
F. Chum	0+	0.020	0.019	0.014	0.016	0.012	0.016
Whitman Lake							
Coho	1+	0.395	0.356	0.353	0.291	0.414	0.346
Chinook	1+	0.454	0.420	0.289	0.349	0.418	0.370
Chinook[3]	1+	0.490	0.450	0.346	0.385	0.450	0.418
Chinook[3]	0+	-----	-----	0.248	-----	-----	0.188

Source: [16,21]

[1] Cost includes debt service, which on average across all species and facilities equals about 30% of total cost.

[2] Age 1+ or yearling smolts are typically released in spring or early summer following 12 to 16 months of post swim-up fry stage feeding in the hatchery. Age 0+ smolts are typically released following 30 to 90 days of post swim-up fry stage feeding in the hatchery or in marine netpens.

[3] Remote release site at Carroll Inlet.

fisheries for salmon are growing in popularity in many countries. Recreational fisheries in the future will play an increasingly important role in the economics of many capture fisheries for salmon.

It has been suggested that enhancement programs for salmon with short freshwater life stages, such as pink or chum salmon, have the best chance for profitable operations.[13,15] The current high production levels and market values for these species that have developed in recent years have begun to change this perception. The average cost of producing different species of juvenile salmon in Southeast Alaska hatcheries over a 5-year period (1988-1992) show an average of $0.016-$0.024 US per chum salmon juvenile and $0.323-$0.418 US per yearling chinook (*O. tshawytscha*) and coho salmon produced (Table 1). In Japan the cost of producing chum salmon during the period 1962-1972 was estimated at 1.94 yen per fry, and 85.4 yen per adult.[17] Roughly a decade later these costs had doubled.[15] In 1987 with an average price of 635 yen per kg paid for adult chum salmon, weighing an average of 3.5kg, this provided a "profit" to the Japanese hatchery programs of about 2200 yen per adult caught.[15]

By 1991, however, a dramatic decline in the price paid for chum salmon to fishermen on Hokkaido had occurred with more than a 20% drop in price from 1990 to 1991.[18]

Prices for chum salmon paid to fishermen in Japan have continued to drop since then with over a 10% decline occurring between 1994 and 1995.[2]

Exvessel values of commercial salmon harvested in Alaska have also fluctuated greatly. During the period 1988-1995, in spite of record catches, annual value of commercially caught salmon in Alaska dropped from over $750 to less than $500 million (Figure 5). Prices paid to

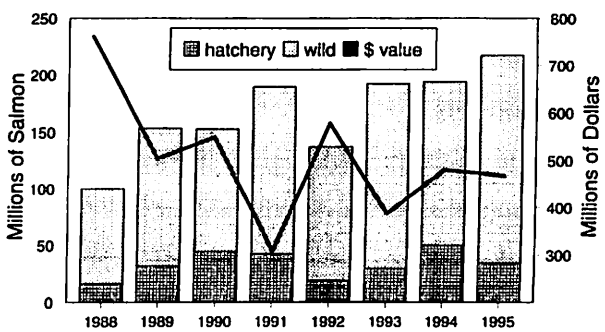

Figure 5. Total numbers of commercially caught wild and hatchery Alaska salmon, 1988-1995, and exvessel value of catch. Hatchery salmon include both common property and cost recovery harvested fish. Source:[19]

fishermen on the grounds in 1988 were at record high levels and the value of one sockeye salmon in Alaska that year was comparable to the $18.00 U.S. value of a barrel of north slope crude oil. Due to world market forces, however, prices for high value sockeye salmon have also declined dramatically. Monthly wholesale prices for Bristol Bay sockeye salmon in Japanese markets since 1990, while highly variable, show a steady declining trend that has reduced prices to roughly half those of a few years earlier (Figure 6).

Average prices paid for purse seine-caught pink salmon in Prince William Sound, Alaska were $1.87 US per kg in 1988 vrs. $0.44 US per kg in 1992, a 76% decline in value over that 5-year period (Table 2). This converts to about $3.00 per fish in 1988 vrs. 70 cents in 1992. Prices have declined further since 1992 and preliminary prices paid for pink salmon in Alaska in 1996 were about $0.20 US per kg

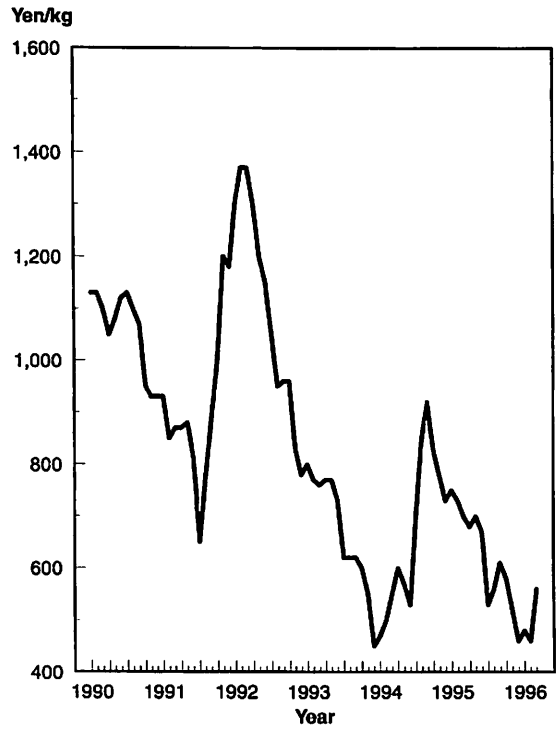

Figure 6. Monthly wholesale price trends in Japanese markets for Bristol Bay sockeye salmon. Source:[20]

(9 cents per pound or 25 cents per fish) when processors were even willing to buy fish. In 1996 there were widespread events throughout Alaska where fishermen were placed on limits on how many pink or chum salmon they could catch with assurance that processors and buyers would purchase the fish. Many millions of available fish went unharvested. In 1996 the primary value of pink and chum salmon in most Alaska fisheries was in the eggs for ikura or sujiko markets.

DISCUSSION

Current economics of world salmon markets have created a two-tiered species alignment that includes moderate-to-low value market species comprised of pink and chum salmon, and premier market species comprised of all farmed salmon and capture fisheries for sockeye, coho, and chinook salmon. Dramatic growth and ready availability of high quality farmed salmon at ever lower cost to consumers,[22] has directly impacted not only the value of other premier market species, but also of pink and chum salmon as well.

The current estimated 1995 world salmon production of 1.544 million mt includes 814 thousand mt of premier market species (all farmed plus capture fisheries for sockeye, coho, and chinook salmon) and 730 thousand mt of pink and chum salmon in the moderate-to-low-value

Table 2. Comparative exvessel prices paid to Alaska commercial salmon fishermen in 1988 and 1992 in the Southeast and Prince William Sound regions by species and harvest gear. Values listed are in U.S. dollars per kilogram and, in parentheses, U.S. dollars per fish.

Species	Harvest Gear	Region: Southeast		Region: Prince William Sound	
		1988	1992	1988	1992
Chinook	Troll	8.39 (70.09)	5.36 (38.15)	[1]	-----
	Purse Seine	5.53 (52.19)	1.94 (14.02)	5.44 (64.91)	2.51 (28.25)
	Gillnet	4.14 (27.44)	2.68 (18.34)	7.24 (86.31)	6.10 (68.54)
Coho	Troll	6.81 (23.79)	3.33 (10.58)	-----	-----
	Purse Seine	4.34 (14.99)	1.65 (5.09)	3.99 (17.47)	1.81 (7.11)
	Gillnet	5.25 (21.15)	2.07 (8.06)	4.42 (19.36)	2.07 (8.61)
Sockeye	Troll	7.61 (18.66)	3.75 (8.48)	-----	-----
	Purse Seine	6.66 (16.31)	3.66 (9.82)	5.77 (16.09)	3.67 (9.96)
	Gillnet	6.81 (20.40)	3.95 (11.28)	6.76 (18.86)	4.76 (12.92)
Chum	Troll	4.25 (13.52)	1.23 (3.60)	-----	-----
	Purse Seine	2.29 (12.06)	1.12 (3.99)	2.34 (9.09)	0.99 (3.51)
	Gillnet	2.55 (11.46)	1.03 (4.18)	2.38 (9.24)	1.53 (5.41)
Pink	Troll	2.16 (2.85)	0.70 (0.89)	-----	-----
	Purse Seine	1.85 (2.68)	0.51 (0.76)	1.87 (2.95)	0.44 (0.66)
	Gillnet	1.87 (3.30)	0.46 (0.80)	1.84 (2.91)	0.45 (0.69)

Source: [16,21]

[1] There is no commercial troll fishery in the Prince William Sound region.

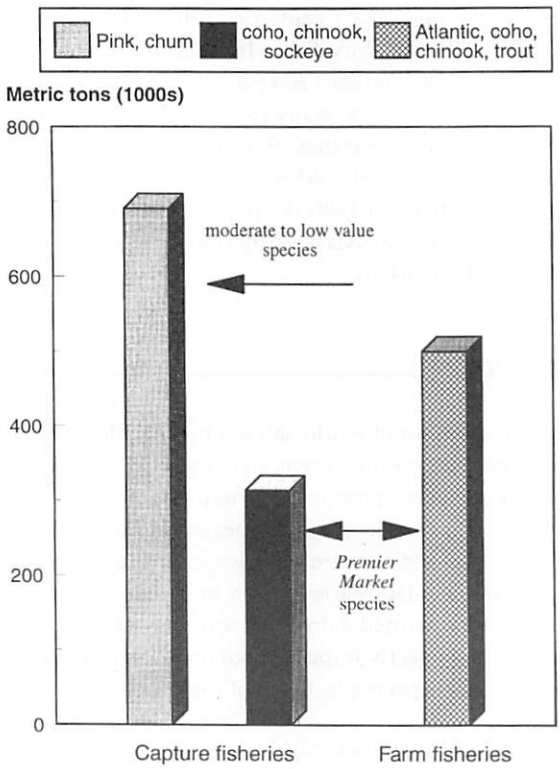

Figure 7. Estimated breakdown of 1995 world salmon production of 1.544 million mt showing amounts produced from farmed and capture fisheries by premier and moderate-to-low value species.

species tier (Figure 7). In the premier market tier, farmed salmon comprise about 60% of the total weight, but represent 70% of the world market value in this group.[23]

This indicates, on average, that farmed salmon have on a per-weight basis slightly higher value in world markets than capture fisheries for sockeye, coho, and chinook salmon.

With direct worldwide competition among premier market species occurring between farmed and capture fisheries resulting in lower prices and increased availability for this group of salmon, the impact on capture fisheries for moderate-to-low value pink and chum salmon has disrupted long-standing established markets and fisheries. The relative value of pink and chum salmon compared to other salmon in world markets is declining and in many fisheries is in a state of flux. Historically most pink and chum salmon caught in North America have been canned. With current record levels of production, along with shifts in market preferences for other salmon products, the economics of the traditional canned salmon industry have come under pressure. Backlogs and carryovers from one season to the next of unsold canned salmon or other traditional products derived from pink and chum salmon have a depressive effect on markets and fisheries for these species. The resulting economic stresses have raised important questions about traditional uses and about managing abundance levels of these salmon.

Suggested solutions to this dilemma have included finding new markets for traditional uses, management to reduce the abundance of these species and, perhaps more importantly, new developments for greater uses with new product forms for pink and chum salmon. Although the traditional salmon canning industry played a historic and important role in development of salmon fisheries throughout North America, this industry has been slow and resistant to changes in addressing contemporary world salmon conditions. Elements of the canning industry, along with some regional fishermen groups have suggested, perhaps naively, that a solution to the current high abundance of pink and chum salmon in Alaska is to reduce or eliminate enhancement programs for these species. This is a poorly thought-out notion that ignores the broader implications of contemporary world issues. Also, it is important to remember that salmon enhancement in Alaska began when wild stocks were at record low levels of abundance in the early 1970s. The primary intent of these programs was to, at least partially, help smooth out the wide cyclic fluctuations in salmon abundance that have periodically occurred throughout the history of Alaska fisheries (Figure 4). Assuming the past cyclic trends in abundance do occur again, and the current abundance levels turn downward, hatchery programs in Alaska and elsewhere will play a vital role in helping industry and fishermen alike withstand future economic hardships of low abundance.

The present worldwide high abundance of pink and chum salmon should be viewed not as a hardship but as an opportunity; an opportunity to find new and better uses for these species. Finding new markets for traditional product forms should certainly be explored in detail. Regarding new markets, it is interesting to note that Japan increased exports of fall chum salmon to China by 400% between the 1994 and 1995 seasons.[24] It is imperative that new non-traditional uses for pink and chum salmon be developed for the efficient utilization of this important, highly renewable protein resource if viable capture fisheries and a processing industry for these species is to remain economically viable.

Some new product forms of modern, user-friendly foods made from pink and chum salmon are being developed. These include various types of salmon hams, sausages, nuggets, spreads, pastes, chips, and condiments. Other new uses also include meal rendering for animal food additatives and gourmet pet foods. Many of these efforts are attempting to find niche markets for speciality products. However, most of these developments to date have been undertaken by small entrepreneurial groups or individuals without capital resources to develop broad markets that would utilize large amounts of pink and chum salmon. Greater involvement and commitment in

developing innovative, new, and different uses of these resources by major seafood processors and food manufacturing corporations is badly needed to help resolve the current dilemma.

The answer to the question title of this report "Are capture fisheries for Pacific salmon in a crisis of abundance" is, paradoxically, both yes and no. Yes, there is an abundance of salmon---not only of moderate-to-low value pink and chum salmon caught in capture fisheries, but also of salmon in general, especially farmed salmon that has been largely responsible for the current two-tiered species alignments in world markets. All indications are that world farmed salmon production will continue to increase in the future and the present total world abundance of salmon is not likely to shift significantly downward soon. The answer to the question about a crisis in capture fisheries for anadromous salmon should be no. The present abundance of these important, renewable, protein resources should be a welcomed bounty from the sea, reflective of sound stock management and fortuitous marine survivals. The real crisis is in the lack of planning and foresight to take advantage of present opportunities to wisely and effectively utilize the current bounty of these fishes.

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CONSERVATION HARVESTING TECHNOLOGY - IS THERE SOME COMMON GROUND BETWEEN AQUACULTURISTS AND FISHING TECHNOLOGISTS ?

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ABSTRACT

With recent trends in fisheries management to identify low risk or precautionary fishery management strategies, the need to identify significant sources of biological waste associated with commercial capture technologies is becoming increasingly important. In addition to landed catches, there are a number of discrete sources of unaccounted fishing mortality that can occur during the capture escape process including discards, avoidance, drop out, ghost fishing and escape mortality. These mortalities may vary by gear type and species, may be immediate or delayed and due to injuries or stressors associated with capture - escape trauma and may include wild fish as well as fish released from enhancement programs.

While various researchers have tried to estimate some categories of unaccounted fishing mortality, a lack of knowledge on the physiological condition prior to capture and how fish cope with fishing induced stressors has been a limiting factor. On the other hand, controlling and reducing the level of stress in fish culture has resulted in increased rates of production, reduced outbreaks of disease and decreased levels of mortality. This paper reviews sources of unaccounted fishing mortality associated with the capture process and suggests that greater cooperation and communication between aquaculturists and fishing technologists can be mutually beneficial. Fish condition during the capture process and quantification and reduction of released juveniles in coastal fishing gears are provided as examples where mutual benefit can be achieved.

INTRODUCTION

The current level of world marine fish catch estimated at 83×10^6 tons and levels of discards, estimated at 27×10^6 tons (33%), suggests that the number of fish being killed globally is in excess of the world's oceans' theoretical potential yield of 100×10^6 tons[1]. FAO[2] have reported that most fish resources are now considered as either fully exploited or over fished. In comparison, the amount of fish and plants produced through aquaculture is continuing to rise and presently amounts to approximately 13.9×10^6 tons (1992 estimates) for all freshwater and marine species. FAO[3] suggests that based on present levels of wild fish harvest being close to the potential yield of fish from all oceans, increases in demand for fish from a growing world population (7 billion by the year 2010) will require fish culture production to increase output from 14 $\times 10^6$ tons to 31×10^6 tons. While such a target seems possible (current increases average about 1×10^6 tons per year), increased rates of wild fish production will place additional stresses on wild fish populations. As a consequence, there has been an increased level of effort to identify, quantify and reduce sources of biological waste associated with commercial capture technologies. Some forms of biological waste such as discards have been the focus of research for many years. Current levels of discards are estimated to be approximately 27×10^6 tons or about 35% of the global catch and represent a significant source of biological waste. In fact, this amount of discards is nearly twice the current level of fish produced from aquaculture globally. In addition to discards, other categories of fishing induced mortalities for each capture technology and fishery remains unclear. There is growing evidence that other unaccounted mortalities such as ghost fishing and escape or release mortality can be significant for some gear types and species of fish.

Quantifying unaccounted fishing induced mortalities is a daunting task to fishing technologists whose expertise is engineering based rather than in fish physiology. In contrast, the aquaculture industry has made good use of

the skills of fish physiologists to identify and reduce environmental and physical factors that induce stress in fish. Clearly, if there is a general desire to reduce levels of biological waste in capture fisheries, there may be some advantages in encouraging cross platform research teams of fishing technologists and aquaculturists to work together. In this light, this paper is a review of the capture process and sources of unaccounted fishing mortality. It describes recent efforts to measure stress and mortality associated with capture and comments on future opportunities for collaborative research.

THE CAPTURE PROCESS

The wide range of capture methods and pre-capture conditions of fish, suggest that the level of injury and stress fish experience during capture and escape may vary significantly over time and space. For example, fish entering an otter trawl generally undergo some degree of forced swimming, confinement, overcrowding and damage due to contact with the fishing gear, debris or other fish as the net is fishing or being retrieved. The amount of time the fish is in the trawl, the towing speed and the depth from which the gear is retrieved, may all affect the degree of injury and level of stress response in fish. Fish caught by other fishing gears may experience a different set of stressors and injuries (for a comprehensive guide to fish catching methods see von Brandt[4]). Nikonorov[5] made some early attempts at defining different stages of the capture process and suggested that capture involves fish passing through zones of influence, action and retention. In this context, the range of influence of the gear is not only where the fish are retained e.g. hook, codend, etc. but also includes parts of the fishing equipment that guide, herd, alarm or scare fish.

Fish injuries

There have been several attempts made to quantify the level of external injuries associated with fish capture including assessment of"life state"charts that record the degree of physical damage, body deformation, blood loss and body movement (see Hoag[6]; Rogers et al.[7]; Main and Sangster[8]). Main and Sangster[8,9], Soldal et al.[10], and Soldal and Isaksen[11] investigated the occurrence of scale removal in gadoids escaping from a demersal trawl; Engas et al.[12] attempted to simulate injuries to cod, haddock and saithe from netting in a tank experiment and Suuronen et al.[13] studied trawl induced injuries in Baltic cod. The occurrence of more traumatic and ultimately fatal injuries has yet to be investigated in detail but the following have been observed in codend escapees: Contusions - On the snout and forehead, the flanks, the operculum edges and caudal fin; Eye Damage - (cataracts); Fin rot - most frequent on the caudal fin (occasionally severe with the whole of the caudal fin degenerating and exposing the end of the spine); Hernia - (including intestinal protrusion from the anal orifice). Damage to the skin, depending on its severity and extent, could result in the loss of osmoregulatory control, mechanical protection, protection from pathogen invasion and sensory reception. The impact of damage to fish eyes will be dependant on the importance of eyesight to the species but may include a reduction in the foraging capability or an increased susceptibility to predators. Severe fin rot may result in lost swimming ability and susceptibility to pathogens. Punctured Abdomen can result from contact with netting, debris and other fish during fishing, gear retrieval and sorting prior to discarding. Mortality is expected to be high due to the initial trauma and associated internal damage as well as making the fish highly susceptible to pathogen / parasitic invasion. Anal hernia is indicative of external pressures on the internal organs of fish and may result from forces applied in the gear (codend, seine bunt etc.) prior to escape or release or incurred during retrieval or sorting of the catch.

Fish stress

Stress is a response mechanism which enables the fish to avoid or overcome potentially threatening, noxious or harmful situations[14]. There are several parts of the fish endocrine system that are sensitive to stress but the predominant endocrine response is an elevation in circulating levels of corticosteroids and catecholamines. A variety of stressors associated with capture and escape have been recorded including; fatigue, damage, confinement, overcrowding and barotrauma. How a fish reacts to one or more stressors will depend upon the fish species, fish condition and the magnitude of the stressors. These will be a function of gear type (trawl, seine, hook, gill net etc.) and mode of operation such as depth, towing speed, retrieval rate etc. (Figure 1). While fish stress responses originate at the endocrine level, their effect may be seen at the metabolic and whole body level (Figure 2).

The most commonly used tests to measure chronic stress associated with capture and handling are plasma cortisol and plasma glucose both of which have limitations. Plasma cortisol levels are commonly used in aquaculture as an index of fish stress[15] and recovery from stress[16] but have only been used on a few fishing gears[17]. Analysing cortisol samples taken from fish at sea is difficult due to the problems in identifying the specific stressors to which the fish have been exposed and obtaining control group fish whose stress accurately reflect the natural condition of the school. Laboratory experiments in which fish were exposed to specific capture stressors (Figure 3-4) have shown that the stress response and mortality can vary according to gear type. Experiments using hook and line

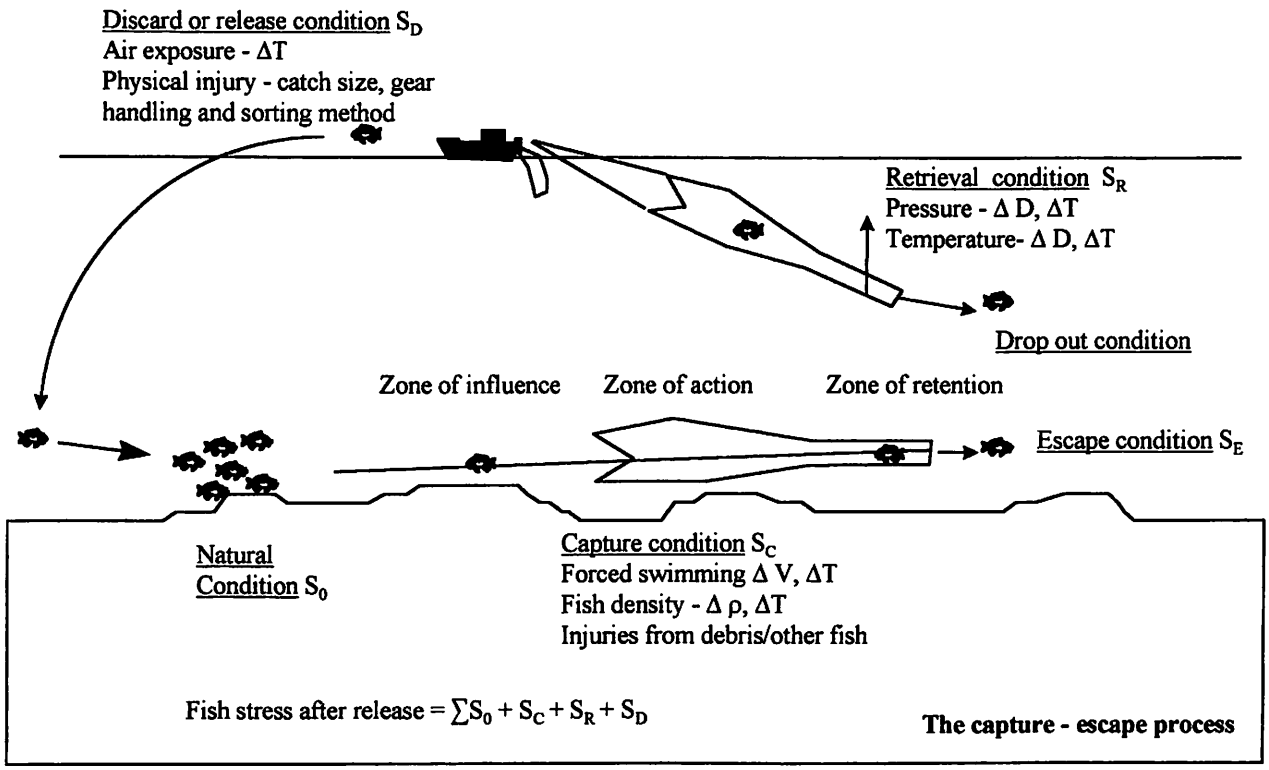

Figure 1. Conceptual diagram of the capture process with fish experiencing a series of fishing gear stressors at different stages of the fishing process.

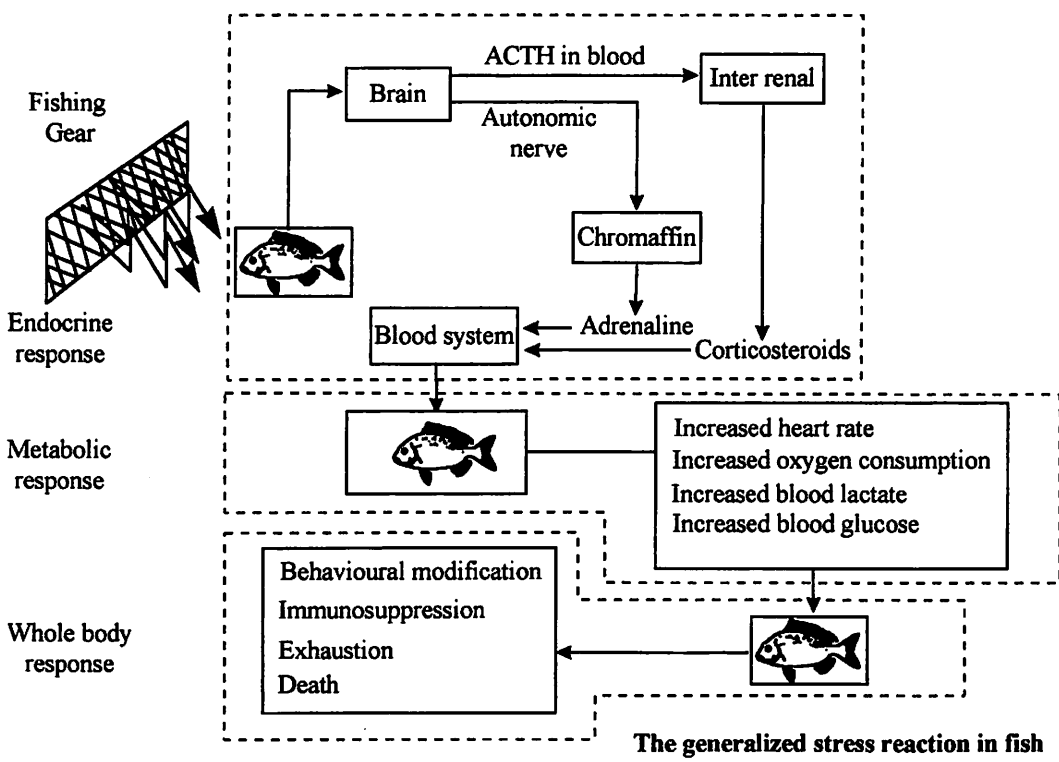

Figure 2. Conceptual diagram stress and fish response at the endocrine, metabolic and whole body level.

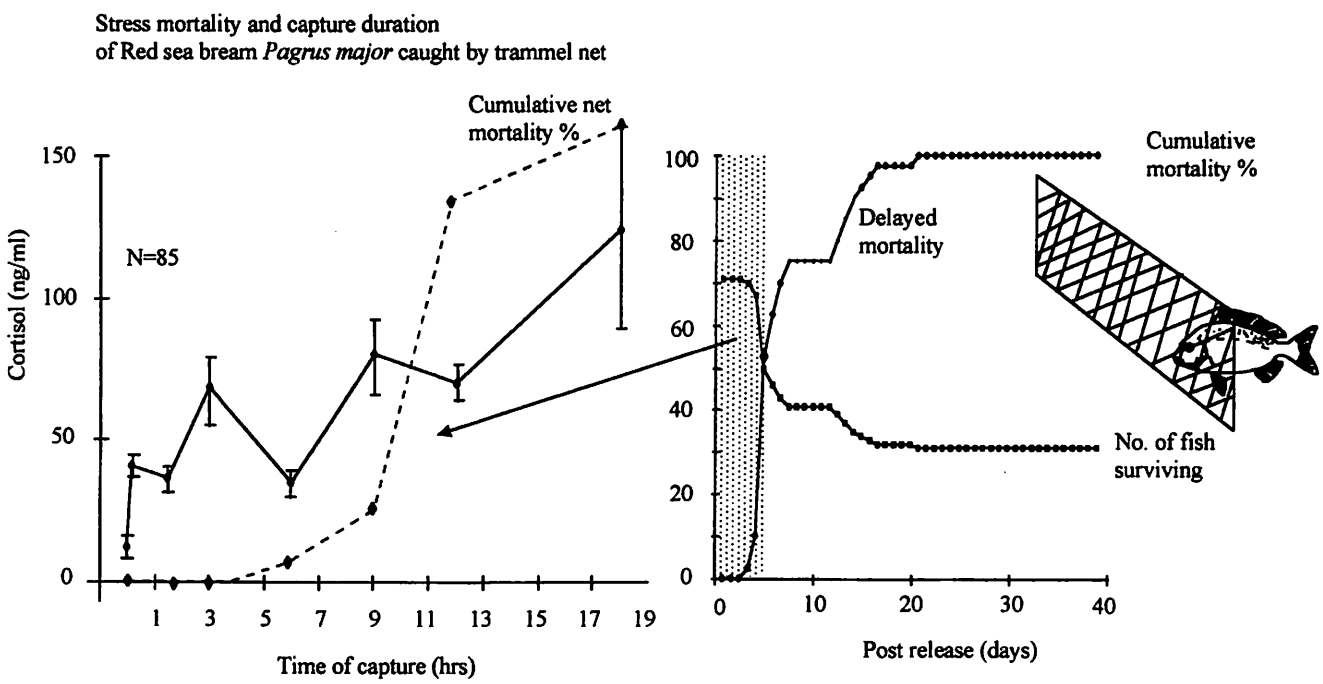

Figure 3. Stress and mortality of fish captured by hook and line as a function of capture duration for trammel net fish (modified from Chopin et al., 1996).

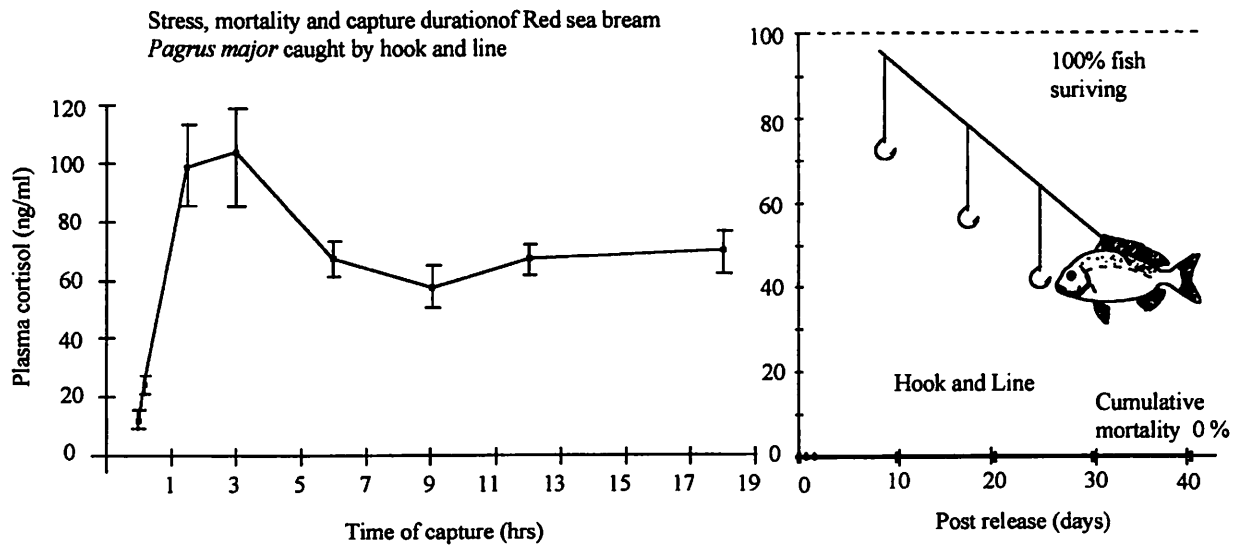

Figure 4. Stress and mortality of fish captured by trammel net as a function of capture duration for hook and line fish (modified from Chopin et al., 1996).

suggest that this capture method may result in low mortalities because fish can adapt to the capture condition. Capture by trammel net results in high mortalities because a cessation in struggling while enmeshed does not reduce the mesh tension around the fish body[18].

SOURCES OF ACCOUNTED AND UNACCOUNTED FISHING MORTALITY

Unaccounted fishing mortalities derives its name from a series of fishing induced mortalities that are not included in estimations of Fishing Mortality F[19]. From a review

of literature on fish condition after escape from fishing gears[20], Chopin and Inoue[21] and Chopin et al.[22] developed a general catch mortality model to describe a variety of discrete types of unaccounted fishing mortality associated with fish capture. Fishing mortality was defined as: The sum of all fishing induced mortalities occurring directly as a result of catch or indirectly as a result of coming into contact with fishing gears. These were expressed by a general fishing mortality equation:

$$F = [F_{CL}+F_{AL}+F_{RL}] + F_B+F_D+F_E+F_G+F_O+F_A+F_P+F_H$$

Where: $[F_{CL}+F_{AL}+F_{RL}]$ represent fishing mortality associated with reported commercial, artisenal and recreational fish landings respectively.

- F_B Illegal and mis-reported-landings
- F_D Mortality associated with discards
- F_E Mortality associated with fish after escape from fishing gear
- F_G Mortality associated with ghost fishing
- F_O Mortality associated with fish passively dropping off or out of fishing gears
- F_A Mortality associated with fish avoiding the fishing gear
- F_P Mortality associated with predation after escape
- F_H Mortality due to changes in habitat associated with fishing

To date, information on the full range of fishing induced mortalities for different species and gear types are not available. The following information is a summary of data collected to date.

Discard mortality estimates

Research directed at understanding the consequences of fish mortality associated with the capture and discard of undersized target species has a long history. However it was not until the latter half of the 20th century that discard studies gained momentum as managers focused their efforts on maximising yield per recruit and achieving it to some extent by improving the size selective properties of the fishing gear. In their development of regional and global bycatch and discard estimates, Alverson et al.[23] estimated that the mean estimate of global discards was 27 $\times 10^6$ metric tonnes (Table 1) based on a catch of 83 $\times$ 10^6 metric.

Escape mortality

Mortalities occurring after escape have been estimated from laboratory experiments using wild and cultured fish, during non commercial fishing trials and by direct observation of commercial fishing gears. Post release observation periods have ranged from 12 h for discard mortalities to in excess of 40 days for otter trawl escape mortalities. Chopin and Arimoto[21] reviewed the level of mortality of fish escaping or released from fishing gears (Table 2) and noted that mortalities could be either immediate or delayed and that fish escaping from fishing gears may die as a direct result of physical damage and stress, or indirectly due to a reduced capacity to escape predators or resist disease.

Experimental methodologies

The methodology of full-scale escape mortality experiments for towed fishing gears consists of three main steps: (a) collection, (b) transportation, and (c) holding and monitoring of the fish. Usually, escapees are collected by a cover / cage mounted around the codend or any selective device that is under investigation. Escapees are often transferred to a remote cage site because of the difficulty of monitoring on the fishing grounds.

The most extensive mortality experiments on ground fish escaping from otter trawls to date have been reported by Sangster and Lehmann[24], and Sangster et al.[25]. Using commercial trawlers, haddock (Melanogrammus aeglifinus L.) and whiting (Merlangus merlangus L.) were studied after escape from 70, 90, 100 and 110mm diamond mesh cod-ends. The mortality rates of haddock and whiting controls were 0%. The mortality rates for the

Table 1. Estimated discard weight by major world region per year (Source: Alverson et al., 1994)

Area	Estimated Discard mt	Area	Estimated Discard mt
Northwest Pacific	9,131,752	Eastern Central Pacific	767,444
Northeast Atlantic	2,671,346	Northwest Atlantic	685,949
West Central Pacific	2,776,726	East Central Atlantic	594,232
Southeast pacific	2,601,640	Mediterranean and Black Sea	564,613
West Central Atlantic	1,600,897	Southwest Pacific	293,394
Western Indian Ocean	1,471,274	Southeast Atlantic	277,730
Northeast Pacific	924,783	Atlantic Antarctic	35,119
Southwest Atlantic	802,884	Indian Ocean Antarctic	10,018
Eastern Indian Ocean	802,189	Pacific Antarctic	109
		TOTAL tonnes	27,012,099

Table 2. Mortality of fish after escape / release from fishing gear (modified from Chopin and Arimoto[20])

Fishing Gear	Species	Mortality %	Comments	Reference
Seine nets	Cod & haddock	0: <10	Fish retrieved at surface	Soldal and Isaksen[11]
Beach seine	Striped Bass	1-17	Mortalities of released fish reduced through improved handling techniques	Dunning et al[31]
Beach seine	Freshwater Drums	84.7	Estimated mortality after release due to stress and injury	Fritz and Johnson[32]
Trawls	Haddock	7 - 78	Fatigue mortality estimated at 0 - 27%	Beamish[33]
Otter trawl	Gadoids		39%-100% surface tagged fish. 12%-65% surface non-tagged fish. 0%-50% bottom tagged fish. 4%-32% bottom non-tagged fish	Hislop and Hemmings[34]
Danishs seine				
Trawl codend	Haddock & whiting	9-27: 10-35	Large variation between species and years	Sangster and Lehmann[27]
Trawls	Melanogrammus sp.		Dead and injured fish found in the wake of the trawl. 163-169 dead fish / hr tow	Zaferman and Serebrov[35]
Trawls	Gadoids	14 - 100	Otter trawls. Large variation in mortality between cages, species and years	Main and Sangster[8]
Trawls	Haddock & whiting	9-27: 10-35	Otter trawl	Anon[36]
Trawls	Cod & haddock	0: 1 - 32	Otter trawl codend	Soldal et al.[10]
Trawls	King and Tanner crab	21-22	Otter trawl. Non target catch	Stevens[37]
Trawls	Lobster	21	Nontarget catch. Mortality varied depending on moult condition	Smith and Howell[38]
Trawls	Atlantic halibut	65	65% mortality after 48h compared to 23% mortality for longline caught fish	Neilson et al. [39]
Trawls	Clupea harengus	85-90: 75-85	Diamond mesh mortality: Sorting grid mortality	Suuronen et al.[40]
Dredges	Pecten sp.	78 - 88	Boat operated scallop dredge. Mortality from gear, predation and disease	McLoughlin et al[41]
Dredges	Placopecten sp.	10 - 17	Boat operated scallop dredge	Caddy, J.F. [42]
Gillnets	Pacific salmon	80 -100	Cumulative mortality in captive fish	Thompson et al,[43]
Gillnets	Pacific salmon	80	Cumulative mortality due to scale damage and stress	Thompson and Hunter[44]
Hooks & Lines	Oncorhynchus sp.	12 - 69	Catch and release mortality estimates	Vincent-Lang et al[45]
Hooks & Lines	Oncorhynchus sp.	34-52: 40-86	Coho salmon: Chinook salmon	Parker et al[46]
Hooks & Lines	Rainbow trout	39: 3 - 5	Hook swallowed corn bait: artificial lure	Barwick, D.H.[47]
Hooks& Lines	Chinook salmon	9 - 32	Trolling. Small fish had higher mortalities	Wertheimer, A.[48]
Hooks & Lines	Pacific salmon	41	Trolling. 34% immediate mortality and 7% delayed mortality.	Milne and Ball[49]

haddock and whiting experimental groups were 36-52% and 40-48% (70mm cod-end), 18-21% and 22-27% (90mm cod-end), 17-27% and 23-33 % (100mm cod-end) and 11-15% and 14-17% (110mm cod-end) respectively.

Full-scale field experiments of fish survival are complex, and many unaccounted or uncontrollable factors may bias results[8,20,24-28]. Recent underwater observations suggest that the small-meshed, hooped cover that is often used to collect escapees may seriously affect the geometry and movements of the codend and thus fish escape behaviour and mortality[28]. Further, there is always some uncertainty about the cause of death of the treatment fish (escapees) even though control group fish held in cages for several weeks have low mortalities. Other problems that have yet to be resolved include: the duration of caging in survival experiments[26]; determining the level of stress associated with physical contact of fish with the cover[10,29-30] and the extremely short time period after starting fishing when fish can be used in the experiment.

To date escape mortality research has tended to focus only on short-term individual effects in the absence of long-term ecological factors such as increased predation risk or impaired growth or reproductive capacity. The inability to collect escapees from long tows and large codend-catches (i.e., under commercial fishing conditions) is probably one of the most serious problems with all present techniques. Other priority areas which require further development are (a) flow-free transportation of

Table 3. Estimated fishing mortality or duration of ghost fishing for pots and gill nets

Species	Gear type	Estimated mortality	Reference
Snow crab	Pot	44.3 crabs per pot = 100 tons	Stevens et al.[50]
Snow crab	Pot	0.5% landed catch	Mallet et al.[51]
Lobster	Pot	670 tonnes	Miller[52]
Sablefish	Pot	326 tonnes	Scarbrooke et al.[53]
Ground fish	Gill net	3600 tonnes fish per annum	Smolowitz[54]
Ground fish	Gill net	74 days: 25 fish + 48 crabs / net	Vienneau and Moriyasu[55]
Herring	Gill net	Nets ghost fish for 7 years	Carr et al.[56]
Dungeness Crab	Pot	10 per pot per year	Breen[57]
Salmon	Gill net	2 years (fish) and 6 years (crabs)	High[58]

escapees to holding cages, (b) capture of adequate numbers and size-classes of non-disturbed control fish, and (c) methodology for a quick and reliable assessment of skin-damages in fish. Since methodological approaches for estimating the survival of codend escapees are still at the development stage, some caution is still needed when interpreting these data.

Ghost fishing mortality

There is no clear definition of ghost fishing and it is difficult in many cases to distinguish between fishing mortalities associated with lost fishing gears, discarded fishing gears and fishing gear debris jettisoned from fishing vessels. For the purpose of this paper, ghost fishing mortality is defined as the number of fish and other animals killed directly or indirectly as a result of injuries and stress incurred due to encountering complete or partial fishing gears that have been lost during the process of setting, fishing or retrieving or cannot be physically retrieved. To date, although ghost fishing is known to occur with some gear types, notably passive fishing gears such as pots, gill nets and trammel nets (Table 3), there has been little systematic research carried out to quantify the full extent of ghost fishing mortality of various fishing gears and there are serious gaps in our knowledge of the short and long term unaccounted fishing mortalities associated with ghost fishing.

DISCUSSION

Fisheries management reforms and technological change over the last thirty years have had some important cosmetic effects in terms of reducing the level of non target species and sizes discarded in some fisheries. However, most of these efforts have been without any direct measure of the reduction in numbers of fish killed and mostly focused on only one category of fishing induced mortality i.e. discards. We try to show that the fish capture process can result in a number of discrete types of fishing mortality

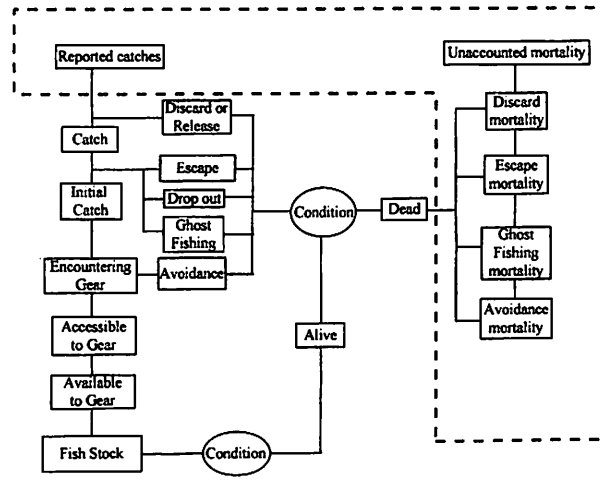

Figure 5. Capture mortality model indicating the various categories of fishing induced mortality

all of which have the potential for being quantified by gear type and fishery (Figure 5). Clearly, there are significant gaps in our understanding of the full extent of fishing induced mortalities for most gear types and species of fish and with respect to experimental methodologies. Only data on discards has been thoroughly reviewed and its impact estimated at 27 million tonnes, is approximately 35% of the reported global catch. Other types of fishing mortality such as drop out mortality, avoidance mortality and the impacts of habitat modification on fish stocks are seldom investigated and need further investigation. Consequently, although we now recognise a variety of different types of fishing induced mortality and perceive some of these to be significant in some fisheries and gear types, they remain to date as an unmeasured component of "natural mortality" M.

Since these unaccounted mortalities are a direct result of coming into contact with fishing gears, a knowledge of how fish condition is affected by trauma and stress during the capture - escape process is essential if any reduction in unaccounted mortality is to be achieved. Clearly there is a role for fish physiologists to play in helping identify the

effect of individual and cumulative stressors on fish health and to be able to measure directly the increased probability of fish survival as a result of changes in capture technology. The opportunity for a new type of commercial fishing physiologist can be seen in two discrete areas. Firstly, there is a need to develop indices of fish condition that are sensitive to environmental and seasonal changes and that allow correlation's to be made between fish condition and probability of mortality. Not only is this of necessity for setting up good control experiments, it has direct benefits in developing more precise measures of natural mortality. Secondly, there is need to quantify the impact of various stages of the capture process on fish health and to measure the effects of any technological improvements designed to reduce unaccounted fishing mortality. For example, many commercial hook and line and pot fisheries require that undersize fish are released. Some measure needs to be made of the probability of survival after gear injury, retrieval from depth, air exposure and handling. Similarly, the cod end mesh size in many trawl fisheries is regulated to allow smaller fish to escape. Such measures have had immediate impact on reducing the level of discards but no measure is made of the condition of fish after escaping from the gear.

If fisheries are to be based on a sustainable basis, there is a need to look beyond reported catches as the best measure of fishing mortality. Research to date has shown that a variety of unaccounted fishing mortalities can occur with all capture technologies and for some gear types and species these may be significant. While dividing fish encountered but not retained into either "alive" or "dead" is useful for statistics, true reductions in unaccounted mortalities requires investigation of fishing and environmental factors that compromise survival. To this end the aquaculture industry with its wealth of experience in physiology and fish behaviour could be of great assistance to the development of our understanding of fish and fishing gear interactions.

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THE NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION (NOAA) LIBRARIES: KEY SOURCES FOR SCIENTIFIC INFORMATION IN AQUACULTURE

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ABSTRACT

This paper describes the NOAA Central Library and other NOAA libraries which have significant collections of aquaculture information. Collectively, the NOAA Library and Information Network, which is comprised of all the NOAA libraries, contains 1.5 million volumes and provides access to hundreds of databases. Some NOAA libraries specialize in meteorological or geophysical information while others concentrate on oceanography and fisheries. This paper focuses on the aquaculture resources available and provides a high-level description of these collections, how to access them, and additional electronic resources.

There are many opportunities to increase communication and cooperation among librarians and information professionals so that scientists will have access to key information. A prime example is the development of the United States/Japan Natural Resources (UJNR) Aquaculture Panel Home Page on the World Wide Web which has been established to provide a gateway to information about the UJNR, such as conference proceedings, researchers' names, and the specialities of each of the UJNR panels. The NOAA Librarians will continue to add information about the panels with the hopes that their Japanese counterparts can do the same. This is a major opportunity for increased cooperation in "information exchange."

INTRODUCTION

The history of the National Oceanic and Atmospheric Administration (NOAA) Central Library stretches back almost two centuries to 1811 when the U.S. Coast and Geodetic Survey Library was created - one of the earliest U.S. government agencies. Publications currently available in the NOAA libraries date from this period. The Survey itself was created only 31 years after the United States became a new independent nation. The United States government decided it needed to survey and map its coastal areas for potential harbors and fishing rights. The first Superintendent of the Coast and Geodetic Survey, F. R. Hassler, established the library which was quickly recognized for its depth and breadth of coverage in fisheries, marine sciences, and other related subjects such as hydrology.

The library has evolved through the years by the addition of comprehensive collections from other government agencies. Large, significant collections from the U. S. Weather Bureau Library and the Fisheries Library were merged with that of the C & GS Library. These historic collections combined with other newly acquired collections, such as the NOAA Law Collection and the NOAA Photo Library, make up today's NOAA Central Library which consists of more than one million books, journals, technical reports, microfiche, microfilm, compact discs, and databases.

NOAA Central Library provides important technical and information services including acquisitions, cataloging, bibliographic control of NOAA publications, online access, training, interlibrary loans, reference, and preservation. Information services numbered 50,000 in 1996. Library clients, or patrons, include government scientists, researchers, economists, and attorneys as well as educators, students, librarians, business people, and many other professionals from outside the government, including individuals from other nations.

In 1995, the NOAA Central Library began charting a new course. Its primary goal continued to be providing the

best service to users in the scientific areas in which NOAA is responsible. In addition, the NOAA Central Library's goals were:

- To provide bibliographic control of NOAA publications.
- To aggressively acquire NOAA publications to ensure that the library becomes the key NOAA source for publication information.
- To preserve NOAA publications and determine which publications have already been preserved in other formats, i.e.microfiche, CD-ROM, or the Internet.
- To solidify agreements with partners in the NOAA Library and Information Network (NLIN) to purchase library publications, such as commercial journals, and services, such as database search services.
- To work with libraries across the nation and the rest of the world to ensure access to a wide array of information sources, especially hard to obtain gray literature. The new "Integrated Library System" will enable patrons to identify which resources are available in the NOAA Central Library as well as the other NOAA libraries. Installed in 1996, this system promises to deliver faster, more coordinated services to users and to the librarians working behind the scenes.
- And finally, to provide electronic access to NOAA publications in cooperation with other NOAA offices and libraries. NOAA libraries have been examining alternative bibliographic methods and have offered new channels of access to information by making databases directly available to library patrons. These databases include Aquatic Sciences and Fisheries Abstracts (ASFA), the NOAA Master Directory, and the Global Change Data and Information System.

NOAA personnel use the World Wide Web to display and link to information and data all over the world. Many NOAA libraries also maintain their own Home Pages and create Internet resources. These library Home Pages can be reached through the NOAA Central Library's Home Page (URL: *http://www.lib.noaa.gov/*). Internet resources provided by NOAA libraries include hypertext links to online publications such as those regularly provided by NOAA's Pacific Marine Environmental Laboratory (PMEL) (URL: *http://www.pmel.noaa.gov /pubs /*). The National Hurricane Center/Tropical Prediction Center Library also provides safety guidelines for natural disasters--tsunamis, floods, and hurricanes--on their Home Page (URL:*http://www. aoml. noaa. gov/general/lib/hurricbro. html*).

The NOAA Central Library not only maintains vast scientific archives, but provides outreach to the community as well with teachers bringing in high school and college students to use the library's resources and business entrepreneurs using the library's resources to research new market segments. Because the National Oceanic and Atmospheric Administration is committed to working with communities to increase knowledge and access to information, the NOAA Central Library and its counterparts work in conjunction with many other libraries and information centers throughout the United States and the world to help all users to acquire information.

Recently, a study of user requests in a one month period produced interesting statistics. Questions from academia numbered 5% while requests from the public comprised 25%. Industry generated the most requests of 32% with 25% of the remainder of questions coming from NOAA and 13% from other government agencies. On a daily basis, library staff receives calls for research and reference which range from the most complicated to the most general. Library staff also produces bibliographies, shares database search results with cooperative publications, and updates the department's Home Page on the World Wide Web (*URL: http:// www.lib.noaa.gov/*).

NOAA Library Services have goals that include current and accurate inventory of the collections and database to guarantee uniformity of bibliographic control/international standards. It monitors the automated catalog, special listings, databases, and new book,journal, CD-ROM, and rare books lists. Plans to provide access to the library catalogs via the Internet are also underway.

The library plans to create an integrated system which, through a wide area network, will connect all of the NOAA Libraries into one system. In connecting the catalog to the Internet, NOAA Central Library will provide access to all NOAA employees as well as many non-federal visitors. In addition, full text access to NOAA resources will be accomplished by digitizing selected documents.

The NOAA Central Library maintains a Photo Library that provides photos, slides, vugraphs, and camera-ready art for presentations, exhibits, and brochures. These materials are available to NOAA and staff offices.

Throughout the year, the Central Library invites NOAA personnel to spend their lunch period in the library for special presentations. These informal lectures, called "brown-bag lunches," bring interested patrons into the library to discuss topics of interest and tour the library facility. This successful program is especially popular with many NOAA scientists. Topics have included: "The Internet: How to Use it," "The Photo Library," and the "New Law Collection." In late October 1996, the Library will present Twister, a recent commercial film about tornadoes, with added commentary from a weather specialist from the National Weather Service.

PARTNERS IN THE NOAA LIBRARY AND INFORMATION NETWORK

The NOAA Library and Information Network (NLIN) has existed for many years. The various libraries work in concert to assist scientists and researchers. If one library has a publication or information needed by another library

to serve a patron, the two libraries share the material as quickly as possible. Many publications in the separate collections are in paper form - books, journals, treatises, law books, and many other materials. Some information is available in electronic form. As more information is produced and accessible electronically, the NOAA's libraries will be able to provide it to patrons more rapidly and efficiently. The NLIN members include:

- NMFS Auke Bay Laboratory Library, Juneau, Alaska (*URL: http://www.wrc.noaa.gov/afsc/abay.html*)
- W. F. Thompson Memorial Library, Kodiak, Alaska (*URL: http://kingfish.ssp.nmfs.gov/facilities/kodiak. html*)
- NMFS La Jolla Laboratory Library, La Jolla, California (*URL: http://swfsc.ucsd.edu / swfsclj.html*)
- NMFS Tiburon Laboratory Library, Tiburon, California (*URL: http://swfsc.ucsd.edu/swfsctb.html*)
- Library - MC5, Boulder, Colorado (*URL: http://www. bldrdoc. gov / library/library.htm*)
- NMFS Milford Laboratory Library, Milford, Connecticut (*URL: http://gopher. wh. whoi. edu / labs/milford. html*)
- NOAA Tropical Prediction Center Library, Miami, Florida NOAA Regional Library, Miami, Florida (*URL: http://www.aoml.noaa.gov / general / lib /*)
- Southeast Fisheries Science Center Library, Miami, Florida (*URL: http://www.sefsc.noaa.gov/mia.html*)
- NMFS Panama City Laboratory Library, Panama City, Florida (*URL: http://www. sefsc. noaa.gov/pan.html*)
- Skidaway Institute of Oceanography Library, Savannah, Georgia
- NMFS Honolulu Laboratory Library, Honolulu, Hawaii (*URL: http://swfsc.ucsd.edu/swfschn.html*)
- NCEP/NESDIS Reading Room, Camp Springs, Maryland
- Oxford Laboratory Library, NMFS SE Fisheries Center, Oxford, Maryland (*URL: http://kingfish. ssp. nmfs. gov:80/facilities/oxford1.html*)
- NMFS Woods Hole Laboratory Library, Woods Hole, Massachusetts (*URL: http://www. mbl. edu / html/LIBRARY / home.html*)
- Great Lakes Environmental Research Laboratory Library, Ann Arbor, Michigan (*URL: http://www.glerl.noaa.gov /*)
- NMFS Pascagoula Facility Library, Pascagoula, Mississippi (*URL: http://www.sefsc.noaa.gov/mis.html*)
- National Weather Service Central Region Library, Kansas City, Missouri (*URL: http://www. crhnwscr.noaa.gov /*)
- ARL/SORD Library, Las Vegas Nevada (*URL: http://www.oar.noaa.gov / ERL / ARL/welcome.html*)
- Lionel A. Watford Library, Highlands, New Jersey (*URL: http://gopher.wh.whoi.edu/labs/sandy.html*)
- Geophysical Fluid Dynamics Laboratory Library, Princeton, New Jersey (*URL: http://www.gfdl.gov/*)
- National Climatic Data Center Library, Asheville, North Carolina (*URL: http://www. ncdc. noaa. gov/ncdc.html*)
- Rice Laboratory, Beaufort, North Carolina (*URL: http://www.sefsc.noaa.gov/bea.html*)
- Atmospheric Sciences Modeling Division Library, Research Triangle Park, North Carolina (*URL: http://www.epa.gov /asmdnerl/)*
- National Severe Storms Laboratory Library, Norman, Oklahoma (*URL: http://www.nssl.uoknor.edu / library / library.html*)
- NMFS Charleston Laboratory Library, Charleston, South Carolina (*URL: http://www. sefsc. noaa. gov/cha.html*)
- Coastal Services Center Library, Charleston, South Carolina
- OAR Turbulence and Diffusion Division Library, Oak Ridge, Tennessee (*URL: http://www.atdd.noaa.gov/*)
- NMFS Galveston Laboratory Library, Galveston, Texas (*URL: http://www.sefsc.noaa.gov/gal.html*)
- NWS Library, Salt Lake City, Utah (*URL: http://www.wrh.noaa.gov /*)
- NOAA Regional Library, Seattle, Washington (*URL: http://www.wrclib.noaa.gov/lib/*)
- National Marine Mammal Laboratory Library, Seattle, Washington (*URL: http://nmml01.afsc.noaa.gov/library/library.htm*)
- Northwest and Alaska Fisheries Science Centers Library, Seattle, WA (*URL: http://research. nwfsc. noaa. gov / library / library.htm*)

RESEARCHING AQUACULTURE

Although researchers should consult the National Agricultural Library, U. S. Department of Agriculture (*URL: http://www.nalusda.gov/*), for many primary materials, the NOAA libraries are also a valuable resource in aquaculture research. The NOAA Libraries which have unique and highly specialized aquaculture materials may be an undiscovered source of knowledge for many researchers. While many of the libraries with resources appropriate to Japanese scholars reside on the west coast of the United States, or off the mainland in Hawaii, several on the U.S.'s east or Gulf Coasts may also prove helpful.

NMFS La Jolla Laboratory Library, LaJolla, CA offers reference services and interlibrary loans in fish biology; fisheries (primarily tuna and Pacific fisheries); marine mammals, biology, and ecology; oceanography; Antarctica; and fishery management. Included in the collection is information on FADS (Fish Aggregation Devices), such as artificial reefs and floating devices that attract marine life. While the library has a limited collection of books on aquaculture, its reference staff can field questions about aquaculture to other resources such as the California

Department of Fish and Game and the Department of Agriculture.

The NMFS Milford Laboratory is actively involved in aquaculture. It was founded as an oyster research facility and hatchery in the 1920s and provided "aquaculture-related" information to the public long before the word "aquaculture" came into common usage. Much of the research in oyster propagation and rearing conducted at Milford in the 1920s through the 1960s is still sought out (see Figure 1). The library receives ongoing requests for the valuable information generated during this period. From the 1960s to the early 1990s the focus switched more to environmental monitoring and analysis, but aquaculture was not completely forgotten. About one half of the library's book holdings are devoted to aquacultural topics and the library currently receives a number of journals and newsletters in the same field. At the present time the Milford Laboratory is actively involved in bay scallop and tautog aquaculture.

In Hawaii, NMFS Honolulu Laboratory Library's collection holds 10,000 books, journals, and reprints, and approximately 500 translations related to Marine fisheries, ecology, mammals and turtles, oceanography, and ichthyology. In recent years, the library has begun extended study of bottom fish and lobster stock assessment. Library staff have also embarked on research in the area of ocean layers and how the level of mixing impacts fish populations.

The National Marine Fisheries Service (NMFS) Woods Hole Laboratory Library in Woods Hole, Massachusetts holds the oldest fisheries collection in the U.S. The library includes a complete collection of the *Bulletins and Reports of the U.S. Fish Commission* and the publications of its successors, the Bureau of Commercial Fisheries and the National Marine Fisheries Services; International Council for the Exploration of the Seas (ICES) documents from 1949 to the present; International Commission for the Northwest Atlantic Fisheries (ICNAF) documents from 1950 to 1979; Northwest Atlantic Fisheries Organization (NAFO) documents from 1979 to the present; and fishing industry periodicals and newspapers. The collection includes 700 periodical volumes, 7,000 other items, and 150 current journal items.

Figure 1. Milford seed oysters

The library's aquaculture holdings include material on stock assessment and population dynamics. Its historical data on fish growth goes back to the late 1800s. The collection also contains information on the commercial fishing industry's impact on stock assessment, ocean dynamics, health quality, and the effects of pollution on fish stocks. Materials on U.S. government regulations and state laws are also available.

The NMFS Charleston Laboratory Library, merging with the Marine Research Library later this year, contains several thousand volumes on fisheries and marine biology. A full one-third of its collection deals with aquaculture. This library, serving National Marine Fisheries, the College of Charleston and the South Carolina Dept of Natural Resources, also contains zoological records on CD-ROM, oceanographic abstracts, and deep sea research.

Salmon, aquaculture, and genetics are the main focus of the Northwest and Alaska Fisheries Science Center Library located in Seattle, WA (see Figure 2). The center library, built in 1935, specializes in resources on salmon stock in the Columbia River, Bering Sea, and Northeast Pacific Ocean. The collection includes approximately 33,000 books and bound journal volumes, 5,000 translations of Russian and Japanese fishery articles, and 6,000 reprints in aquaculture, marine science, fisheries, and ecology.

NMFS Pascagoula Facility Library has a smaller collection of 1,200 books, 2,150 journals (163 different titles), and 200 reprints relating to marine science and seafood technology. The library uses two research vessels that go out into the Atlantic to develop appropriate gear for shrimp fishing. In developing the trawling efficiency device, the facility library has responded to U.S. laws

Figure 2. This picture was photographed in an American Indian aquaculture site in the Pacific Northwest. It depicts culchless (without shell) spat (baby oyster) after 6 months of growth.

protecting sea turtles.

Seattle's NOAA Regional Library, while concentrating on the subjects of meteorology, oceanography, geophysics, and mathematics, also aids students and scientists of aquaculture by providing access to the Aquatic Science and Fisheries Abstracts (ASFA) database and directions to other sources in the area like the Ocean Library at the University of Washington. Like Seattle's NOAA Regional Library, many of the NOAA libraries are able to provide researchers with access to electronic resources. The Seattle Regional Library's Home Page offers electronic access to aquaculture resources (URL: *http://www.wrclib.noaa. gov/lib/aqua.html*).

ELECTRONIC RESOURCES

Electronics resources, most available via the Internet, extend the ability of researchers to obtain aquaculture information as well as NOAA Library Services' ability to provide aquaculture information. Some useful electronic resources are the NOAA Directory, NODC's Taxonomic Code, and the United States/Japan Natural Resources Aquaculture Panel (UJNR) Home Page.

The Environmental Services Data Directory as well as the NOAA Directory are both available on the NOAA Environmental Information Services page (*URL: http://www.esdim.noaa.gov/*). In the section "How can I find data available at NOAA?", the researcher selects NOAA Data Set Catalog and then Full-text Search Only. At that point the user has several choices to select whatever data he/she needs. The format in which to view the data set descriptions include: Generic Hypertext (HTML), Government Information Locator Service (GILS), Federal Geographic Data Committee Metadata Standard, and Native or Original Format (DIF or NEDRES) (see Appendix B).

The National Oceanographic Data Center's Taxonomic Code is also a useful tool for scientists. Data from Version 8.0 of the NODC Taxonomic Code can be found on the Interagency Taxonomic Information System (ITIS) Home Page (*URL: http://www.itis.usda.gov/itis/*). The ITIS is the result of a partnership of federal agencies collaborating with systematists in the federal, state and private sectors to provide scientifically credible taxonomic information. It includes documented taxonomic information of flora and fauna from both aquatic and terrestrial habitats, primarily in North America. The ITIS is currently under development by NODC, the U.S. Environmental Protection Agency, the U.S. Geological Survey (including the former National Biological Service [NBS]), the U.S. Department of Agriculture and several other agencies. Searches can be performed through the ITIS home page using either the common (vernacular) name, the scientific name, or the taxonomic serial number (TSN). The search will yield other names that have been used historically for the organism (synonymy), plus the hierarchical placement of that name (see Appendix C).

NOAA Central Library is host to the UJNR Home Page (*URL: http://www.lib.noaa.gov/japan/ujnr.html*) which has been established to provide a gateway to information about the UJNR. This home page has the potential to develop into a very useful resource on aquaculture. Currently, it is able to provide information on conference proceedings, researchers' names, and the specialities of each of the UJNR panels. Conference proceedings, as well as other aquaculture materials, will soon be available in full-text.

COOPERATIVE OPPORTUNITIES FOR THE FUTURE

This paper has provided an overview of some of NOAA's capabilities and interests in the field of aquaculture. We recognize the critical need for information to be available for many uses -- for stock assessments, economic analyses, and scientific study. NOAA librarians look forward to working with our Japanese colleagues to further the availability of aquaculture information. We have a joint opportunity to enrich the scientific communities knowledge of this valuable resource.

APPENDIX A: NOAA LIBRARY AND INFORMATION SERVICES DIVISION HISTORY

1811 Coast & Geodetic Library (CGL) created
1871 Weather Bureau Library (WBL) created - U.S. Fisheries Commission Library created
1965 CGL became Geophysical Science Library (GSL), WBL became Atmospheric Sciences Library (ASL)
1967 GSL & ASL became one library
1970 NOAA established as a Federal agency
1971 Library and Information Services Division (LISD), a part of NOAA's new structure, was established
1989 LISD became a new division of the National Oceanographic Data Center (NODC) in Washington, D.C.
1994 LISD appointed as a U.S. Government Depository Library
1995 NOAA Photo Library collection added to LISD
1996 NOAA Law Collection added to LISD

APPENDIX B: ENVIRONMENTAL SERVICES DATA DIRECTORY OR NOAA DIRECTORY SEARCH

URL: *http://www.esdim.noaa.gov/*

1. Go to the NOAA EIS Home Page.
2. Look for "How can I find data available at NOAA",

click on *NOAA Data Set Catalog*.

3. On the NOAA Data Set Catalog Page, click *Full-text Search Only*.
4. On the Full-text Search Page, select which database you would like to search; then type in your search query. Click on the *Start Search* button to begin the search.
5. At the next page, select the format in which you wish to view the data set descriptions. The options are:
   - Generic Hypertext (HTML)
   - Government Information Locator Service (GILS)
   - Federal Geographic Data Committee Metadata Standard
   - Native or Original Format (DIF or NEDRES)

   Click on *Retrieve Documents* to display your search results.

APPENDIX C: SEARCHING FOR INFORMATION FROM THE NODC TAXONOMIC CODE, VERSION 8.0

URL: http://www.itis.usda.gov/itis/

Searches can be performed using either the common (vernacular) name, the scientific name, or the taxonomic serial number (TSN). The search will lead to other names that have been used historically for the organism. The currently accepted scientific name will also be identified. (There are about 250,000 TSNs on the Version 8.0 CD-ROM.)

1. Go to the ITIS: Taxonomy for Biodiversity (Interagency Taxonomic Information System) Home Page.
2. Click on Access Database.
3. On the Data Access Page, click on Query the ITIS Database.
4. On the Database Query page, select the Kingdom and type of input (Scientific Name, Vernacular Name, TSN (Taxonomic Serial Number) an type your search in the text box..
5. Click *Submit* to perform the search.

Ordering information for NODC Taxonomic Code, Version 8.0, CD-ROM:

Mail: National Oceanographic Data Center
User Services Group
NOAA/NESDIS E/OC1
SSMC3, 4th Floor
1315 East-West Highway
Silver Spring, Maryland 20910-3282
Telephone: (301) 713-3277
FAX: (301) 713-3302
Internet: services@nodc.noaa.gov

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APPENDIX

Agenda for the 25th Japan-U.S. Joint Meeting on the UJNR Aquaculture Panel

October 15 (Tuesday)
- p.m. Arrive in Yokohama
  (National Research Institute of Fisheries Science)
  Pre-Business meeting

October 16 (Wednesday)
- Business Meeting
- Symposium "Biodiversity and Aquaculture - for Sustainable Development"
- Welcome party hosted by the Japanese chairman of the UJNR Aquaculture Panel

October 17 (Thursday)
- Symposium continued

October 18 (Friday)
- a.m. Travel to Yokosuka
- p.m. Visit Japan Marine Science and Technology Center (JAMSTEC) in Yokosuka
  Special Session I
  "Discussions on Effective Utilization and Environmental Preservation of the Coastal Water, and Discussions on Activities of Marine Stock Enhancement in Kanagawa Prefecture"
  Dinner cruise on Kanazawa-Hakkei, Yokohama

October 19 (Saturday)
- a.m. Travel to the Izu Peninsula
  Visit Suzuhiro "Kamaboko" (boiled fish paste) manufactory in Odawara
- p.m. Visit Japan Sea-Farming Association, Minami-Izu Center

October 20 (Sunday)
- Travel to Tokyo via Izu-Hakone National Park

October 21 (Monday)
- a.m. Visit Central Research Institute of Electrical Power Industry, Abiko Research Laboratory in Abiko, Chiba
  Free discussion
- p.m. Visit National Research Institute of Fisheries Engineering (NRIFE) in Hasaki, Ibaraki
  Information exchange on offshore culture
  Free discussion
  Welcome party hosted by the NRIFE

October 22 (Tuesday)
- a.m. Short tour of the Choshi Peninsula
- p.m. Visit Ibaraki Prefectural Sea-Farming Center (IPSC)
  Special Session II
  "Discussions on the stock enhancement program of Japanese flounder in Ibaraki"
  Travel to Tsukuba
  Welcome Party hosted by Dr. Fukusho of JIRCAS

October 23 (Wednesday)
- a.m. Visit Tsukuba Agriculture Research Hall
  Visit Japan International Research Center for Agricultural Sciences (JIRCAS)
- p.m. Visit Maruha Corporation Central Research Institute
  Travel to Tokyo

October 24 (Thursday)
- Tours and visits around Tokyo including Tsukiji Fish Market
- Farewell Party at Ginza Lion Brasserie

October 25 (Friday)
- Depart from Tokyo

SYMPOSIUM PROGRAM (held at NRIFS, Yokohama)

"Biodiversity and Aquaculture - for Sustainable Development"

October 16 (Wednesday)

11:00 - 11:15

Opening Remarks — *Masanori Azeta*, *Japanese Chairman, UJNR Aquaculture Panel*

I Problems of Aquaculture Field
(Chairmen: Dr. James J. Sullivan & Dr. Kazumi Hosoya)

11:15 - 11:35

1. Relationship between red tide outbreaks, phytoplankton diversity and phytoplankton sequence in embayments
   Takuji Uchida, Yukihiko Matsuyama (Nansei Nat'l Res. Inst.) and Tsuneo Honjo (Kyusyu Univ.)*

11:35 - 11:55

2. Potential threat of eco-estrogens and other environmental endocrine disruptors to fish
   Howard A. Bern (Univ. California, Berkeley)

11:55 - 12:15

3. Impact of mariculture on the spatial and temporal patterns of the macrobenthos in Gokasho Bay
   Hisashi Yokoyama, Katsuyuki Abo, Masaya Toyokawa, Satoru Toda and Shigeya Yamamoto (Nat'l Res. Inst. Aquacult.)*

..... ***Lunch Brake*** .....

13:30 - 13:50

4. Impacts of shellfish introductions on local ecosystems
   Bruce Barber (Univ. Maine)

13:50 - 14:10

5. The functions of production and purification by diverse organisms on an intertidal flat
   Katsuyuki Sasaki (Nat'l Res. Inst. Fish. Sci.)

II Problems of Intensive Aquaculture Production
(Chairmen: Dr. Robert R. Stickney & Dr. Nobuhiko Taniguchi)

14:10 - 14:30

1. Research on genetic improvement of aquacultured species in Japan
   Katsuhiko T. Wada (Nat'l Res. Inst. Aquacult.)

14:30 - 14:50

2. The use of captive broodstocks for gene conservation of salmon in the western United States
   Michael H. Schiewe, Thomas A. Flaggs and Barry Berejikian (Northwest Fish. Sci. Cent., NMFS)*

14:50 - 15:10

3. Genetic evaluation of qualitative and quantitative traits of hatchery stocks for aquaculture in Red Sea Bream
   Nobuhiko Taniguchi, Motohiro Takagi and Seiji Matumoto (Kochi Univ.)*

..... ***Coffee Brake*** .....

15:30 - 15:50

4. The present situation and points at issue in breeding by chromosomal set manipulation in Hirame Paralichthys olivaceus
   Kazuo Tabata and Akira Mizuta (Hyogo Pref. Fish. Exp. St.)*

15:50 - 16:10

5. Introduction of nonindigenous species for aquaculture in Japan
   Kazunori Fujii (Nat'l Res. Inst. Aquacult.)

16:10 - 1630

6. Development of submerged offshore net-pen technology in New Hampshire, USA

Barbaros Celikkol (Univ. New Hampshire)

October 17 (Thursday)

III Problems of Extensive Aquaculture Production-1
(Chairmen: Dr. Howard A. Bern & Dr. Yo Yamashita)

9:30 - 9:50

1. Comparison of the genetic variation between wild fish and artificial seeds
   Tetsuo Fujii (Japan Sea Nat'l Fish. Res. Inst.) and Mutsumi Nishida (Fukui Pref. Univ.)*

9:50 - 10:10

2. Risk taking release from non-native stocks of the ayu
   Kei'ichiro Iguchi (Nat'l Res. Inst. Fish. Sci.)

10:10 - 10:30

3. The use of bioenergetic measurements to estimate prey consumption, nutritional status and thermal habitat requirements for marine organisms reared in the sea
   A. J. Paul (Univ. Alaska)

10:30 - 10:50

4. Quality of fish for release: Behavioural approach
   Katsumi Tsukamoto (Ocean Res. Inst., Univ. Tokyo)

Coffee Brake

11:10 - 11:30

5. A natural tracking method for summer flounder, Paralychthys dentatus, in stock enhancement programs
   Christopher G. Duffy, George C.Nardi (Great Bay Aquafarms)*

11:30 - 11:50

6. The gulf of marine Atlantic cod stock complex, patterns of distribution and movement of the Sheepscot Bay substock
   Herbert C. Perkins, Stanley B. Chenoweth, Richard W. Langton (Maine Depart. Mar. Resources)*

11:50 - 12:10

7. An ecophysiological model for predicting performance and impacts of released fish
   John M. Miller (North Carolina State Univ.)

12:10 - 12:30

8. Stock structure of the flounder in the Japan Sea in relation to the stock enhancement
   Masaru Tanaka (Kyoto Univ.)

Lunch Brake

IV Problems of Extensive Aquaculture Production-2
(Chairmen: Dr. Conrad Mahnken & Dr. Katsuhiko T. Wada)

13:30 - 13:50

1. Overview of an experimental stock enhancement program for red drum in South Carolina
   Theodore I. J. Smith, Wallace E. Jenkins and Michael R. Denson (South Carolina Mar. Resources Res. Inst.)*

13:50 - 14:10

2. Review of marine finfish stock enhancement in Hawaii
   Cheng Sheng Lee (Oceanic Inst.)

14:10 - 14:30

3. Introduction of exotic species and biohazard
   Kazumi Hosoya (Nat'l Res. Inst. Fish. Sci.)

14:30 - 14:50

4. Preservation of endangered species and regeneration of extinct species in fish using biotechnology
   Hiroshi Onozato (Shinshu Univ.)

Coffee Brake

15:10 - 15:30

5. Are capture fisheries for Pacific salmon in a crisis of abundance?
   William Heard (Auke Bay Lab., NMFS)

15:30 - 15:50

6. Marine enhancement programs-Implementation planning
   Robert R. Stickney (Texas A & M Univ.)

15:50 - 16:10

7. Governmental research funding and policy for biotechnology on aquaculture
   Kenji Matsumoto (Research Div., Fish. Agency)

16:10 - 16:30

8. Conservation harvesting technology - Is there some common ground between aquacuturists and fishing technologists?
   Frank Chopin, Yoshihiro Inoue and Yoshiki Matsushita (Nat'l Res. Inst. Fish. Engineer.)*

16:30 - 16:50

9. The national oceanic and atmospheric administration (NOAA) libraries: A key source for scientific information in biodiversity and aquaculture
   Carol B. Watts (NOAA Libraries)

Coffee Brake

17:10 - 17:45

V Discussion
(Chairmen: Dr. James J. McVey & Dr. Masanori Azeta)

17:45 - 18:00

Closing Remarks

James P. McVey
U.S. Chairman, UJNR Aquaculture Panel

*: Speaker

SPECIAL SESSION I PROGRAM (held at JAMSTEC, Yokosuka)

"Discussions on Effective Utilization and Environmental Preservation of the Coastal Water, and Discussions on Activities of Marine Stock Enhancement in Kanagawa Prefecture"
(Chairman: Dr. Nagahisa Uki)

October 18 (Friday)

13:00-13:10
Opening Remarks — *Shiro Uno*, *Director, Coastal Research Department, JAMSTEC*

13:00-13:10
1. Introductory speech
Masanori Azeta (NRIA, Japanese Chairman, UJNR Aquaculture Panel)

13:10-13:25
2. Development of an underwater elevator system for monitoring environments in Sendai Bay
Kimiaki Kudo (2nd Group Senior Researcher, JAMSTEC)

13:25-13:40
3. Development and evaluation of a submersible platform for mariculture
Mineo Okamoto (4th Group Senior Researcher, JAMSTEC)

13:40-14:05
4. Development of a seafloor platform for culturing the Japanese rock oysters
Yukihisa Washio (1st Group Associate Researcher, JAMSTEC)

14:05-14:20
5. Using deep seawater for biological production
Toshimitsu Nakashima (1st Group Associate Researcher, JAMSTEC)

..... ***Coffee Break*** .....

14:30-15:00
6. Present state of the flounder stock enhancement project
Ryosei Nakamura (Kanagawa Prefectural Experimental Station)

15:00-15:20
7. On our strategy and activities of enhancement for the flounder in the United States
James P.McVey (Program Director, National Sea Grant Program, Aquaculture)

15:20-15:50
8. Free discussion

15:50-16:00
Closing Remarks — *James. P. McVey*, *U.S. Chairman, UJNR Aquaculture Panel*

SPECIAL SESSION II PROGRAM (held at NRIFE, Hasaki)

"Discussions on the Enhancement Program of Japanese Flounder in Ibaraki Prefecture"
(Chairman: Mr. Junya Higano)

October 22 (Tuesday)

14:00-14:10

Opening Remarks — *Kyoichi Ishikawa, Managing Director of IPSC*

14:10-14:40

1. On the present seafarming activities of Japanese flounder at Ibaraki Seafarming Center
   Kenji Sakae (Ibaraki Prefectural Seafarming Association)

14:40-15:10

2. Resources management of Japanese flounder in coastal waters of Ibaraki Prefecture
   Akira Nihira (Ibaraki Prefectural Fisheries Experimental Station)

15:10-15:40

3. A study on the improvement of planning and design of artificial nursery ground of Japanese flounder
   Katsunori Kimoto (NRIFE)

15:40-16:00

4. Free discussion

16:00-16:10

Closing Remarks — *James. P. McVey, U.S. Chairman, UJNR Aquaculture Panel*