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Biological Control and Improvement of Salmon and Advanced Concept of the Technology for Aquaculture

Proceedings of the 23 rd Japan-U.S.A. Joint Meeting on Aquaculture in Mie, Japan, November 17-18, 1994 followed by its Satellite Symposium in Niigata November 21, 1994.

Edited by Masanori Azeta, Kazumi Hosoya,
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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Kunizo Tanaka, Japan
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Under the U.S.-Japan Cooperative Program in Natural Resources (UJNR)

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PREFACE

The United States and Japanese counterpart panels on aquaculture were formed in 1969 under the United States-Japan Cooperative Program in Natural Resources (UJNR). The panels currently include specialists drawn from the federal departments and Sea Grant Programs most concerned with aquaculture. Charged with exploring and developing bilateral cooperation, the panels have focused their efforts on exchanging information related to aquaculture which could be of benefit to both countries.

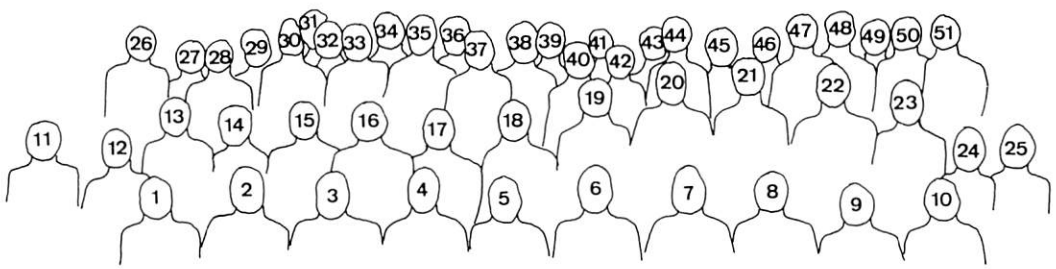
The UJNR began with the Third Cabinet-Level Meeting of the Joint United States-Japan Committee on Trade and Economic Affairs in January 1964. In addition to aquaculture, current subjects in the program include desalination of seawater, toxic microorganisms, air pollution, energy, forage crops, national 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 23rd U.S.-Japan Aquaculture Panel Symposium was held in Ise City, Mie, Japan from November 17 to 18, 1994, focusing on 'Biological Control and Improvement of Salmon'. It was also followed by a satellite Symposium on 'Advanced Concept of the Technology for Aquaculture' in Niigata, November 21, 1994 during a field trip from Mie through Nikko to Tokyo. Both symposia were organized and programmed by Munehico Iwata, Katsuhiko Ito and other UJNR staff members on the Japanese side. Editorial work has been achieved with the great help of NRIA staff members, particularly by Nagahisa Uki, Kouichi Kawamura and Kimika Yamamoto.

Kunizo Tanaka - 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 the symposium of the U.S.-Japan Aquaculture Panel Meeting in the United States-Japan Cooperative Program in Natural Resources (UJNR). It was edited by the UJNR/Japan Aquaculture Panel, following the reagreement between U.S.-Japan Panels in the 23rd business meeting that the proceedings should be produced by the nation which hosted the meeting. The proceedings before the 20th symposium edited by the UJNR/U.S. Aquaculture Panel on American side had been published in the series of NOAA Technical Report (NMFS). It has renamed as UJNR Technical Report since the 22nd symposium in Homer, Alaska, 1993.



Participants in 23rd Japan-USA Joint Meeting on Aquaculture, held in Ise City, Mie Japan, November 17-18, 1994.

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IMPROVEMENT IN THE MARINE SURVIVAL OF CHUM SALMON BY THE CONTROL OF PROTOZOAN INFECTIONS

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ABSTRACT

The potential impact of the ectoparasitic protozoan *Ichthyobodo necator* on marine survival of juvenile chum salmon *(Oncorhynchus keta)* was evaluated by laboratory and field surveys. The infection experiments indicated that the parasite infections caused severe epidermal destruction and drastically reduced the seawater tolerance of juvenile chum salmon due to osmoregulatory breakdown. A control experiment was conducted at Yoichi Hatchery along the Yoichi River in western Hokkaido, where the parasite infections annually occurred, resulting in the reduced seawater adaptability of juvenile chum salmon. A formalin bath (250 ppm for 1 h) eliminated the attaching parasites and recovered the seawater adaptability of salmon juveniles before release from the hatchery. The number of adult salmon returns significantly increased in the Yoichi River after the control of parasites. These results suggest that *Ichthyobodo* infections cause high mortality in juvenile chum salmon soon after they migrate to the ocean. The control of parasite infections is essential to improve the marine survival of infected fish.

INTRODUCTION

The early ocean life of anadromous salmonids may be a critical period in their life history, but the possible causes of mortality are poorly understood. Many factors affect the early marine survival of salmonids, such as predation, food limitation, density-dependence, ocean conditions, and physiological conditions (Pearcy 1992). Diseases also may be a factor that affects survival of salmonids in the ocean. Although various pathogens cause disease conditions among hatchery-reared salmonids, their impact in natural water are poorly understood.

The objectives of the present study are to evaluate the potential impact of the parasitic protozoan *Ichthyobodo necator* on marine survival of juvenile chum salmon *(Oncorhynchus keta)* by laboratory and field surveys, and to examine control methods to improve the marine survival of infected fish.

RESULTS

PARASITE

Ichthyobodo necator is a parasitic flagellate infecting the skin and gills of wild and hatchery-reared salmonids and many other fish species (Robertson 1985). This small single cell parasite is originally a freshwater species, but can reproduce in the marine environment as a result of its adaptation to anadromous salmonid hosts (Urawa and Kusakari 1990). The parasite is commonly distributed in the Northern Hemisphere, and occurs in 30–40% of salmon hatcheries in northern Japan (Urawa 1992a). The mortality of *Ichthyobodo*-infected fish is usually low in fresh water, although high mortality occasionally occurs when infections are combined with environmental stress such as high rearing density or poor water quality (Urawa 1995).

IMPACT OF PARASITE ON MARINE SURVIVAL OF SALMON

A transmission experiment was conducted in the laboratory to estimate the influence of parasite infections on survival of juvenile chum salmon in fresh water and seawater (Urawa 1993). The parasite density increased 2 weeks after infection, reached a peak at week 6, and then decreased gradually by week 10 (Figure 1). Parasites attached to the epidermal cells, and caused sloughing of the entire epidermis above the basal layer (Figure 2) when the parasite density increased between 4 and 6 weeks after infection (Urawa 1992b). The cumulative mortality in fresh water for 10 weeks was

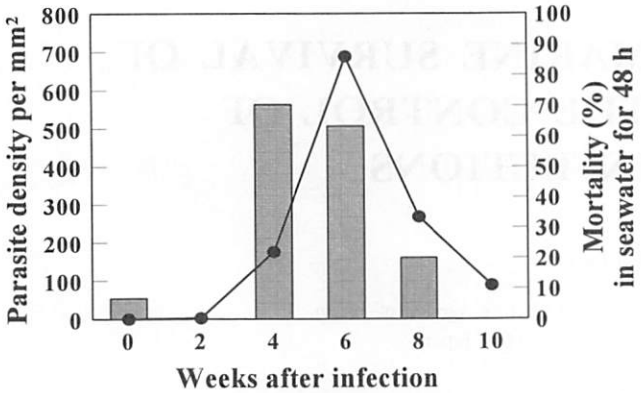

Figure 1. Biweekly changes in the density of *Ichthyobodo necator* on the skin of juvenile chum salmon in fresh water (solid circles) and the mortality of juveniles transferred in seawater (salinity 33‰) within 48 hours (shaded columns).

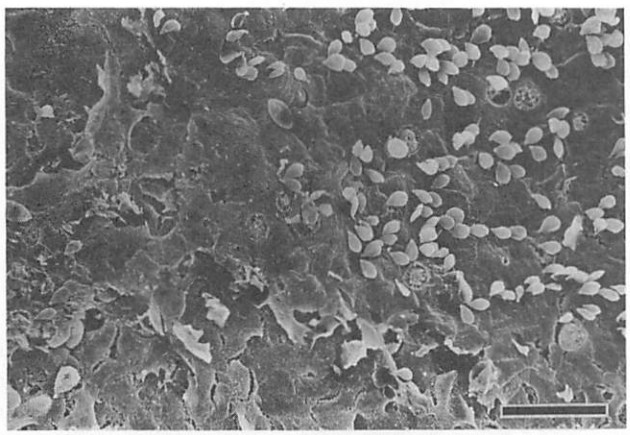

Figure 2. Scanning electron micrograph of the skin surface of juvenile chum salmon infected with *Ichthyobodo necator*. The upper layer of epidermis sloughed off, on which no parasite appeared, in contrast to high infections on the remaining epidermis regions. Bar=40 μm.

12% in infected group, and 2% in the controls. Biweekly seawater challenge tests (salinity 33‰ for 48 h) indicated that 60–70% of infected fish died in seawater between weeks 4 and 6 (Figure 1). When high mortalities were recorded in infected fish between weeks 4 and 6, the serum chloride concentrations of infected fish were significantly lower in fresh water and higher in seawater than those of the controls. In the controls, serum chloride concentration gradually reached seawater adaptive levels of about 130 meq/l within 48 hours after seawater transfer. In infected fish, however, it suddenly increased to 180 meq/l within 3 hours after seawater transfer and continued at much higher levels over the next 4 days (Figure 3). This initial sharp rise in serum chloride levels was followed by death in many infected fish, indicating that acute dehydration occurred in seawater due to an

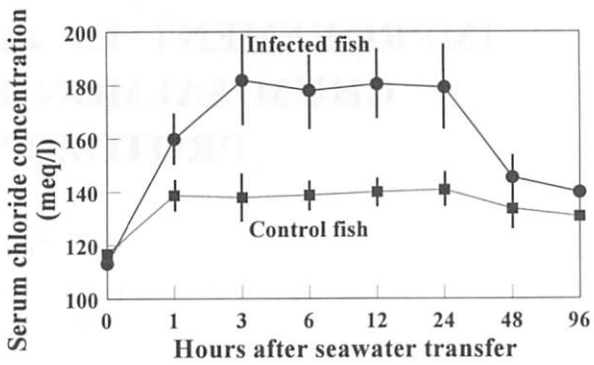

Figure 3. Changes in the serum chloride concentration of *Ichthyobodo* infected and control chum salmon juveniles held in fresh water following exposure to seawater at week 6. Bars indicate SD.

osmoregulatory disorder of injured skin. These results suggest that the parasite has a high potential to cause mass mortalities in juvenile salmon soon after their ocean entry.

CONTROL OF PARASITE INFECTIONS

To verify the hypothesis that *I. necator* induces high marine mortality of juvenile chum salmon, a control experiment was conducted in the spring of 1987 at Yoichi Hatchery along the Yoichi River in western Hokkaido, where the parasite infections annually occurred, resulting in low survivals of chum salmon (Urawa 1992c). This hatchery is located 4 km upstream from the estuary of the Yoichi River, and juvenile salmon enter the sea soon after release from the hatchery. Biweekly observations indicated that the parasite density increased in fry stage, and the seawater survival rate reduced to 70% (Figure 4). However, a formalin bath (250 ppm for 1 h) eliminated the attaching parasites and recovered the seawater adaptability of salmon juveniles within 4 weeks post-treatment (Figure 5). Thus, approximately 9 million juvenile chum salmon were released by the middle of April after the complete treatment.

Although the number of juvenile chum salmon released from the hatchery increased gradually year by year, adult returns had been very scanty (less than 4,000 fish) until 1988 (Figure 6). However, adult returns significantly increased in the Yoichi River in 1989–91, the returning years of the 1987 salmon juveniles treated with formalin bath. These increased salmon returns are largely a consequence of the improved early marine survival of juvenile salmon due to the control of *Ichthyobodo* infections.

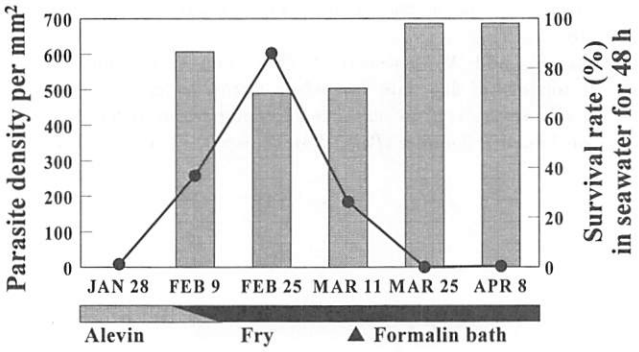

Figure 4. Seasonal changes in the mean density of *Ichthyobodo necator* (solid circles) and seawater survival (salinity 33‰ for 48 h; shaded columns) in juvenile chum salmon reared in the Yoichi Hatchery in 1987. Shaded and solid bars under the abscissa indicate the alevin and fry stages of salmon, respectively. Arrow denotes the date when a formalin bath (250 ppm for 1 h) was conducted.

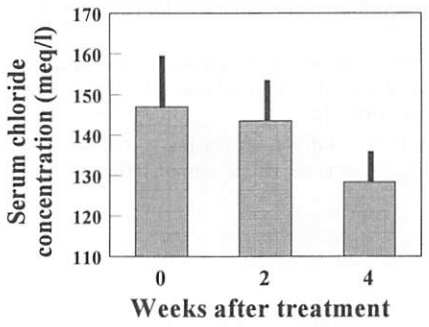

Figure 5. Changes in the serum chloride concentration of juvenile chum salmon held in seawater (salinity 33‰) for 48 h after a treatment with formalin bath. Bars indicate SD.

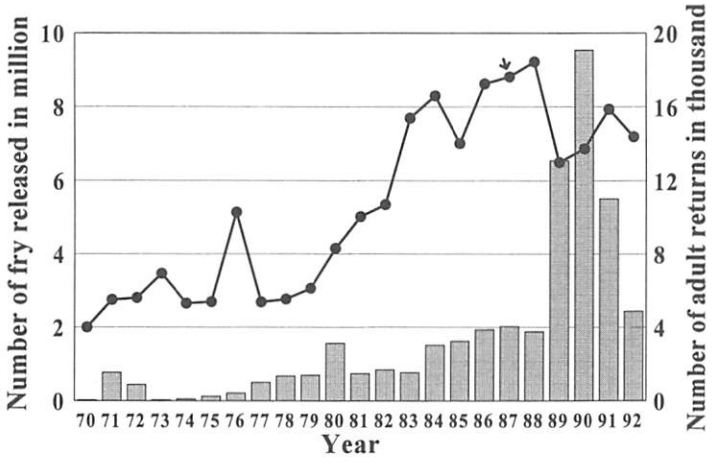

Figure 6. Annual Changes in the number of juvenile chum salmon released (solid circles) and adult returns (shaded columns) in the Yoichi River between 1970 and 1992. Arrow indicates juvenile chum salmon treated with formalin bath before release in 1987.

CONCLUSIONS

The present study has demonstrated that *Ichthyobodo necator* infections become a factor causing high mortality among juvenile chum salmon in natural water, especially when they enter the coastal ocean. Consequently, the control of parasite infections is essential to improve the marine survival of infected fish. The parasite is common in hatchery-reared salmonids as well as in wild fishes. Hatchery-reared salmonids should be examined for the parasite, and infected fish should be treated by one month prior to their release.

Natural mortalities of anadromous salmonids may be also caused by other pathogenic organisms including bacteria (e.g. *Renibacterium salmoninarum* and *Flavobacterium branchiophila*), protozoans (e.g. *Ceratomyxa shasta* and *Chilodonella piscicola*), and metazoan parasites (e.g. *Nanophyetus salmincola*). Further studies are requested to understand the influence of these pathogens on the survival of anadromous salmonids in the ocean. The present methods that combine laboratory infection experiments with field population survey may be effective for evaluating the possible impact of pathogens.

LITERATURE CITED

Pearcy, W. G. 1992. Ocean ecology of North Pacific salmonids. Univ. Washington Press, Seattle. 179 p.

Robertson, D. A. 1985. A review of *Ichthyobodo necator* (Henneguy, 1883) an important and damaging parasite. In Recent advances in aquaculture, Vol. 2. Edited by J. F. Muir and R. J. Roberts. Croom Helm, London. pp. 1–30.

Urawa, S. 1992a. Host range and geographical distribution of the ectoparasitic protozoans *Ichthyobodo necator*, *Trichodina truttae* and *Chilodonella piscicola* on hatchery-reared salmonids in northern Japan. Sci. Rep. Hokkaido Salmon Hatchery 46: 175–203.

Urawa, S. 1992b. Epidermal responses of chum salmon (*Oncorhynchus keta*) fry to the ectoparasitic flagellate *Ichthyobodo necator*. Can. J. Zool. 70: 1567–1575.

Urawa, S. 1992c. The pathobiology of ectoparasitic protozoans on hatchery-reared Pacific salmon. Ph.D. thesis, Hiroshima Univ. 148 p.

Urawa, S. 1993. Effects of *Ichthyobodo necator* infections on seawater survival of juvenile chum salmon (*Oncorhynchus keta*). Aquaculture 110: 101–110.

Urawa, S. 1995. Effects of rearing conditions on growth and mortality of juvenile chum salmon (*Oncorhynchus keta*) infected with *Ichthyobodo necator*. Can. J. Fish. Aquat. Sci. 52 (Suppl. 1): 18–23.

Urawa, S., and M. Kusakari. 1990. The survivability of the ectoparasitic flagellate *Ichthyobodo necator* on chum salmon fry (*Oncorhynchus keta*) in seawater and comparison to *Ichthyobodo* sp. on Japanese flounder (*Paralichthys olivaceus*). J. Parasitol. 76: 33–40.

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RESEARCH ON CHINOOK SALMON (ONCORHYNCHUS TSHAWYTSCHA) STOCK STRUCTURE USING MICROSATELLITE DNA

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ABSTRACT

Preliminary microsatellite studies in chinook salmon (Oncorhynchus tshawytscha) have revealed six highly polymorphic loci which indicated strong potential for stock discrimination. Winter, fall and late-fall stocks of the Sacramento river, California, USA had 3.6, 6.8 and 5.0 mean number of alleles per locus and 32.8%, 53.9% and 63.5% average observed heterozygosity respectively. Genotypes of offspring from controlled crosses demonstrate that this marker type has simple Mendelian codominant inheritance. Although the same alleles were common in more than one stock, allele and genotype frequencies demonstrated strong stock dependancy. Isolation and characterization of more microsatellite loci for chinook to increase power for stock discrimination appears well justified given this strong potential.

Historically, the water drainage system of California's central valley (Western USA) has hosted abundant chinook salmon (Oncorhynchus tshawytscha) stocks. Less than one hundred years ago, the upper reaches of the Sacramento river was famous for spawning runs which had peaks of several hundreds of thousands of gravid fish, four times a year (Winter, Spring, Fall and Late Fall). Serious decline in the number of returning adults in recent years has raised great concern, particularly for Winter and Spring runs. In 1994 Winter run chinook of the Sacramento river became listed as an endangered species.

Given the potential to modify water management policies in order to protect particular stocks, a search for molecular genetic markers for discrimination between alternate stocks has become a strong priority. We have been characterizing nuclear encoded short tandem repeat DNA polymorphism (microsatellites) as candidate molecular markers with strong potential, primarily due to their high mutation rate of 10-3 to 10-4 gametes per generation (Queller et al 1993; Weissenbach et al 1992; Hearne et al 1992; Edwards et al 1991). This is 3-5 orders of magnitude greater than rates of electrophoretically detectable amino acid substitutions or nucleotide substitutions in mitochondrial or nuclear DNA. Microsatellites therefore, are more likely to reflect recent genetic divergences and are thus suited to the task of resolving the population complex of the Sacramento river chinook salmon.

Observations from samples derived from a captive broodstock for winter run chinook and from wild fish including alternate stocks, have confirmed that these markers are indeed very abundant, highly polymorphic and rapidly typed in large population surveys. Most importantly, they demonstrate simple mendelian inheritance as codominant markers (Table 1), and are easily amplified using the polymerase chain reaction. These features greatly facilitate analysis and enhance the rigor of inferences that can be drawn from data.

The first striking feature of the preliminary results for the different wild runs (Table 2) is the significantly lower genetic diversity in winter- vs fall- or late-fall samples, as reflected in mean numbers of alleles per locus, 3.6 vs 6.8 and 5.0, and average observed heterozygosity, 32.8% vs 53.9% or 63.5%, respectively (Table 3, Figure 1). Lower genetic diversity across all six loci for winter-run compared to fall- or late-fall run might be explained by the hypothesis that winter-run experienced a population bottleneck during the construction of Shasta Dam. Whatever its cause, lower genetic diversity of winter-run may aid in discriminating it from other stocks.

The second pertinent feature of our preliminary results is that although the same alleles are common in more than one stock (Table 2), allele and genotype frequency profiles show strong stock dependency (Figure 1). For example at the 2A locus which demonstrates the most marked stock dependency, genotype J-J occurs in 68% of winter-run, 3% of fall-run and none of the late-fall run sampled (Table 4).

Table 1. Genotype frequencies from a broodstock family formed by mating dam 8 by sire C at three loci. Chi-square tests indicate that the observed frequencies do not have any significant deviation from what would be expected assuming simple Mendelian transmission.

Locus	Parent genotypes		Offspring genotypes				κ^2	P
G85	BD × AD		AB	AD	BD	DD		
		Observed:	8	5	8	5		
		Expected:	6.5	6.5	6.5	6.5		
							1.385	0.84
2A	CC × AD		AC	CD				
		Observed:	13	12:				
		Expected:	12.5	12.5				
							0.04	0.84
B4	DE × BC		BD	BE	CD	CE		
		Observed:	4	6	8	7		
		Expected:	6.5	6.5	6.5	6.5		
							1.385	0.71

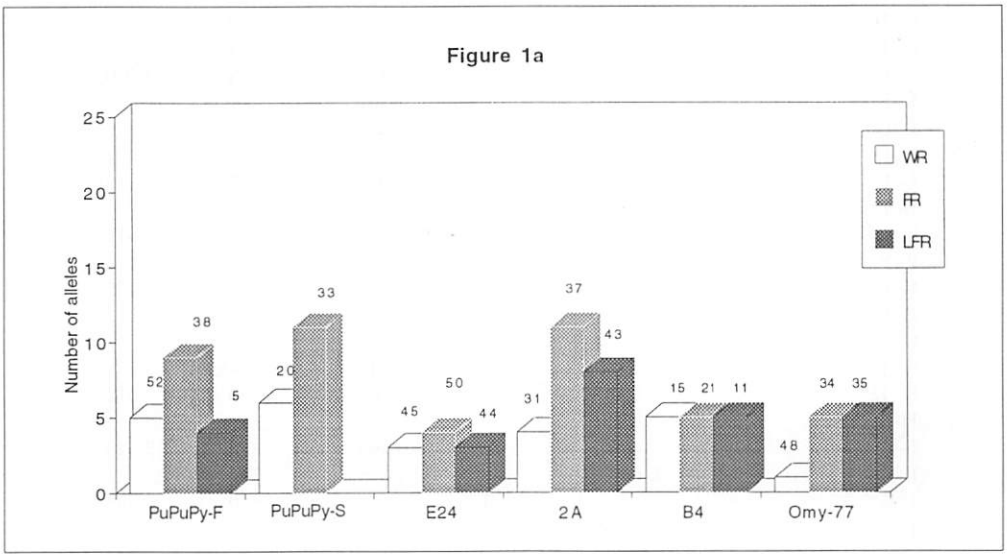
Table 2. Allelic frequencies in samples of Sacramento River winter-run, fall-run and late-fall run chinook for 5 microsatellite loci. N is the number of individuals typed.

	Population				Population		
Locus	WR	FR	LFR	*Locus*	WR	FR	LFR
PuPuPy-F				*2A*			
(N)	52	38	5	(N)	31	37	43
A	.019	.013	.000	A	.000	.054	.093
B	.010	.026	.100	B	.000	.041	.035
C	.000	.039	.100	C	.000	.054	.070
D	.000	.000	.000	D	.000	.014	.000
E	.606	.645	.600	E	.032	.257	.407
F	.356	.145	.200	F	.000	.068	.070
G	.000	.026	.000	G	.000	.068	.000
H	.010	.053	.000	H	.016	.054	.023
I	.000	.000	.000	I	.129	.311	.291
J	.000	.000	.000	J	.823	.068	.000
K	.000	.039	.000	K	.000	.014	.012
L	.000	.013	.000				
				B4			
PuPuPy-S				(N)	15	21	11
(N)	20	33	—	A	.233	.214	.273
A	.000	.030	—	B	.433	.214	.136
B	.025	.015	—	C	.233	.214	.136
C	.100	.076	—	D	.067	.333	.318
D	.000	.061	—	E	.033	.024	.136
E	.025	.076	—				
F	.125	.242	—	*OMY77*			
G	.550	.364	—	(N)	48	34	35
H	.000	.015	—	A	.000	.029	.057
I	.000	.061	—	B	.000	.059	.043
J	.175	.045	—	C	1.000	.882	.829
K	.000	.015	—	D	.000	.015	.029
				E	.000	.015	.043
E24							
(N)	45	50	44				
A	.678	.650	.648				
B	.000	.020	.000				
C	.311	.290	.330				
D	.011	.040	.023				

Table 3. Genetic variability in samples of Sacramento River winter-run,fall-run and late-fall run chinook for 5 microsatellite loci; (standard errors in parentheses).

Population	Mean sample size per locus	Mean no. of alleles per locus	Percentage of loci polymorphic*	Mean heterozygosity	
				Direct-count	HdyWbg expected**
1. '91–'93 WR	38.2 (6.8)	3.6 (.7)	80.0 (.089)	.328 (.120)	.398
2. '93 FR	36.0 (4.6)	6.8 (1.4)	100.0 (.091)	.539 (.108)	.574
3. '93 LFR	27.6 (8.2)	5.0 (.8)	100.0 (.102)	.635 (.090)	.595

* A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.99.
** Unbiased estimate (see Nei, 1978).



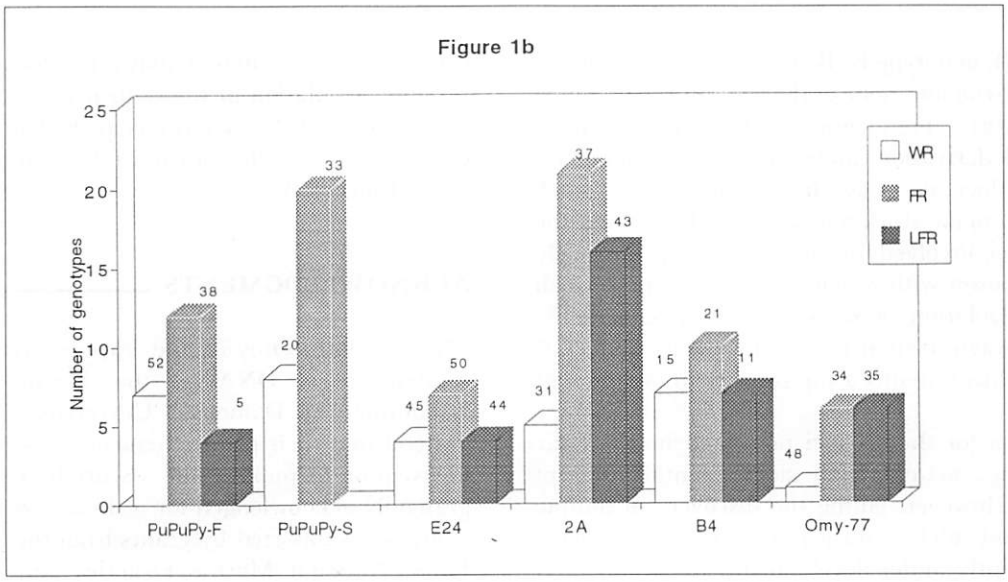


Figure 1. Number of observed alleles (a) and genotypes (b) for Sacramento River chinook at six microsatellite loci. Numbers above bars indicate sample size.

Table 4. Observed and expected genotype frequencies for the most divergent microsatellite locus, 2a for Sacramento River winter-run, fall-run, and late-fall run chinook salmon. Only genotypes occuring at least once in any run are listed. Dashes indicate a genotype for an allele that does not occur in that run.

	WR		FR		LFR	
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
A–A	–		0	.082	1	.329
A–C	–		1	.219	0	.565
A–D	–		1	.055	–	
A–E	–		0	1.041	4	3.294
A–F	–		0	.274	1	.565
A–G	–		1	.274	–	
A–H	–		1	.219	0	.188
A–I	–		0	1.260	1	2.353
B–E	–		1	.781	2	1.235
B–F	–		1	.205	1	.212
B–I	–		1	.945	0	.882
C–E	–		2	1.041	2	2.471
C–H	–		1	.219	0	.141
C–I	–		0	1.260	4	1.765
E–E	0	.016	3	2.342	5	7.000
E–F	–		0	1.301	1	2.471
E–G	–		2	1.301	–	
E–H	0	.033	0	1.041	2	.824
E–I	0	.262	7	5.986	13	10.294
E–J	2	1.672	1	1.301	–	
E–K	–		0	.260	1	.412
F–F	–		0	.137	1	.176
F–I	–		3	1.575	1	1.765
F–J	–		1	.342	–	
G–I	–		1	1.575	–	
G–J	–		1	.342	–	
H–H	0	.000	1	.082	0	.012
H–J	1	.836	0	.274	–	
I–I	1	.459	5	3.466	3	3.529
I–J	6	6.689	0	1.575	–	
I–K	–		1	.315	0	.294
J–J	21	20.902	1	.137	–	

Likewise, at B4, genotype B–B occurs in 27% of winter-run, 1% of fall-run and none of the late-fall run sampled (data not shown). Frequency data from individuals of unknown stock derivation can be matched to a data base formed from loci such as these which show stock dependency to make stock allocations. Increasing the number of loci involved in such matching obviously increases the power with which one is able to make such allocations. Isolation of more chinook microsatellite loci, their characterization for the four runs and PCR optimization thus remains a top priority in our present research.

Clearly, data for the six loci presented here are far from providing unambiguous stock identification of individuals. However, failing the discovery of unique stock dependent alleles and genotypes for new microsatellites currently under development, we submit that differences between stocks in frequencies at shared microsatellite loci, enable an estimation of the relative contributions of different spawning stocks to the mixed juvenile population in the central valley delta. Statistical procedures known as mixed stock analysis are well developed and verified for this type of application (Pella and Milner 1987).

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LITERATURE CITED

Edwards, A., A. Civitello, H. A. Hammond, and C. T. Caskey. 1991. DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. Am. J. Hum. Genet. 49: 746–756.

Hearne, C. M., S. Ghosh, and J. A. Todd. 1992. Microsatellites for linkage analysis of genetic traits. Trends Genet. 8: 288–294.

Nei, M. 1978. Estimation of average heterozygosity and geneitc distance from a small number of individuals. Genetics, Austin Tex. 89: 583–590.

Pella, J. J. and G. B. Milner. 1987. Use of genetic markers in stock composition analysis. In: Rayman, N and Utter, F (eds) Population Genetics and Fishery Management. Washington Sea Grant, University of Washington Press, Seattle.

Queller, D. C., J. E. Strassmann, and C. R. Hughes. 1993. Microsatellites and kinship. Trends Ecol. Evol. 8: 285–288 + centerpage.

Weissenbach, J., G. Gyapay, D. Dib, A. Vignal, and others. 1992. A 2nd-generation linkage map of the human genome. Nature 359: 794–801.

Bull. Natl. Res. Inst. Aquacult., Suppl. 2: 11-15 (1996)

POPULATION DYNAMICS AND STOCK MANAGEMENT OF HATCHERY-REARED SALMONS IN JAPAN

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ABSTRACT

Life histories of sockeye (*Oncorhynchus nerka*) and chum salmon (*O. keta*), which feed on zooplankton and conduct school-behavior, are affected by the population density dependence related to intraspecific competition. This competition affects not only the individual growth rate and maturation but also the distribution and migration of populations. Life histories of sockeye and chum salmon are considered to be subject to a conditional strategy. These salmonids take residence or migration tactics by available resource. This strategy conforms to the ideal free distribution model. On the other hand, intraspecific competition leads to population density effects such as changes in individual growth and age composition in chum salmon population. The biomass condition of sockeye and chum salmon populations is controlled by the density effect as well as by the environmental change in habitat. In salmon enhancement production, therefore, it is extremely important to control a optimum of population system by clarifying the mechanism of population regulation such as density-dependent effect, and by monitoring biological characters of population such as migration pattern, body size, age composition, and fecundity.

INTRODUCTION

Since the 1980s, the biomass of Pacific salmons (genus *Oncorhynchus*) has been increasing throughout the North Pacific Ocean. According to FAO fisheries statistics, the annual catch of Pacific salmons in the area averaged 0.7 million tonnes during 1981-1991, and in 1992 exceeded 0.9 million tonnes, which surpassed the historical high. The biomass of chum (*O. keta*) in Japan, sockeye (*O. nerka*) in North America, and pink salmon (*O. gorbuscha*) in North America and Japan is increasing. This increase is thought to have coincided with favorable oceanic conditions and successful artificial enhancement programs (Kaeriyama 1989; Pearcy 1992; Brodeur and Ware 1992; Beamish and Bouillon 1993).

However, with this increase in population size, individual growth reduction has been observed for many Pacific salmon populations, such as Bristol Bay sockeye (Rogers and Ruggerone 1993), Japanese chum (Kaeriyama 1989, Ishida *et al.* 1993), and Prince William Sound pink salmon (Thomas and Mathisen 1993). These findings suggest that salmon research from a viewpoint of population ecology is very important in order to carry out the stock management and effective artificial enhancement program of Pacific salmon.

The purpose of this paper is to clarify population-density-dependent effects as intraspecific competition on Pacific salmon populations.

MIGRATION AND RESIDENCE

Life histories of sockeye and chum salmons, which feed on zooplankton and conduct school-behavior, are affected by the population density dependence related to intraspecific competition. This competition affects not only the individual growth and maturation but also the distribution and migration of populations.

LACUSTRINE SOCKEYE SALMON

Lacustrine sockeye salmon released from a hatchery, have been established in Lake Shikotsu. The sockeye salmon have two tactics of residence and migration in their life-history strategy. That is, they usually remain in the lake as a resident type while they sufficiently obtain their resources such as food and habitat, whereas a part of the population migrates seaward as smolt after one or two years in the lake when they do not have enough those resources (Kaeriyama 1991). Figure 1 shows a relationship between population size and smolt rate (number of smolts / population size) of Lake Shikotsu sockeye salmon. Smolts in 1984–1986 (145± 4 mm) were significantly smaller ($P<0.001$) in fork length and higher ($P<0.001$) in smolt rate than those in

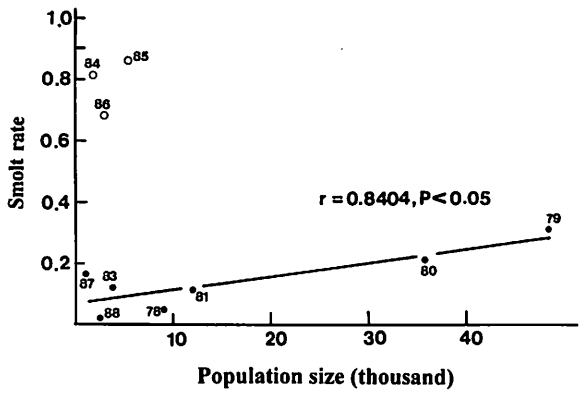

Figure 1. Relationship between population size (P) and smolt rate (S) of sockeye salmon in Lake Shikotsu: $S = 0.0000045P + 0.0699$. The population size shows total numbers of smolt and adult, and the smolt rate represents the number of smolt per population size in a population. Mean fork lengths of smolt populations are 183 ± 15 mm in 1978–1983 and 1987–1988, and 145 ± 4 mm in 1984–1986. Data are obtained from Kaeriyama (1991).

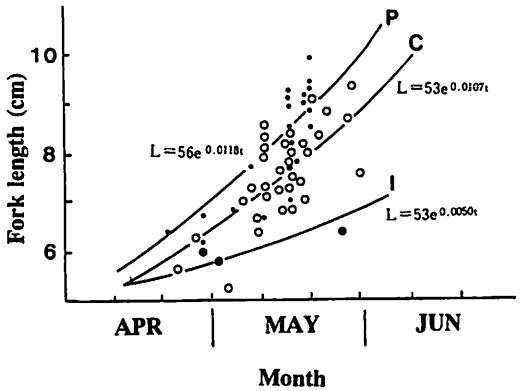

Figure 2. Growth curves of marked juvenile chum salmon released from the Kitakami River (Kaeriyama 1986). P (•): offshore migration type (food: pelagic organisms), C (○): neritic residence type (food: coastal zooplankton), I (●): shore residence type (food: terrestrial insects).

other years (183 ± 15 mm). Significant positive relationship between population size and smolt rate was observed except for 1984-1986 populations ($r = 0.8404$, $P < 0.05$).

These results indicate that 1) their residence or seaward migration may be affected both by the population density and resource condition in the lake, and that 2) they may migrate seaward when they do not fully benefit from their resources.

JUVENILE CHUM SALMON

More than 90% of chum salmon are produced by an artificial enhancement program in Japan. Wild populations are not much. For both hatchery-reared and wild juvenile chum salmon, the migration pattern is controlled by effects of "prior residence" in spawning area or in released site and "precedent migration" in rivers and at sea (Kaeriyama 1986).

Juvenile chum salmon migrate from early spring to early summer in Japan. Only a few fry emerging initially from spawning redds or released from hatchery in early spring remain in rivers for several months with low specific growth rate (effect of prior residence). However, numerous juveniles migrate seaward immediately after the emergence or the release. Those juveniles remain in the coastal sea for a time and migrate offshore. This offshore migration is usually preceded by larger juveniles, which have higher growth rate, than by others in a population (effect of precedent migration).

Figure 2 shows that larger marked juveniles released from the Kitakami River in the spring of 1983 began to migrate offshore and to eat pelagic organisms earlier

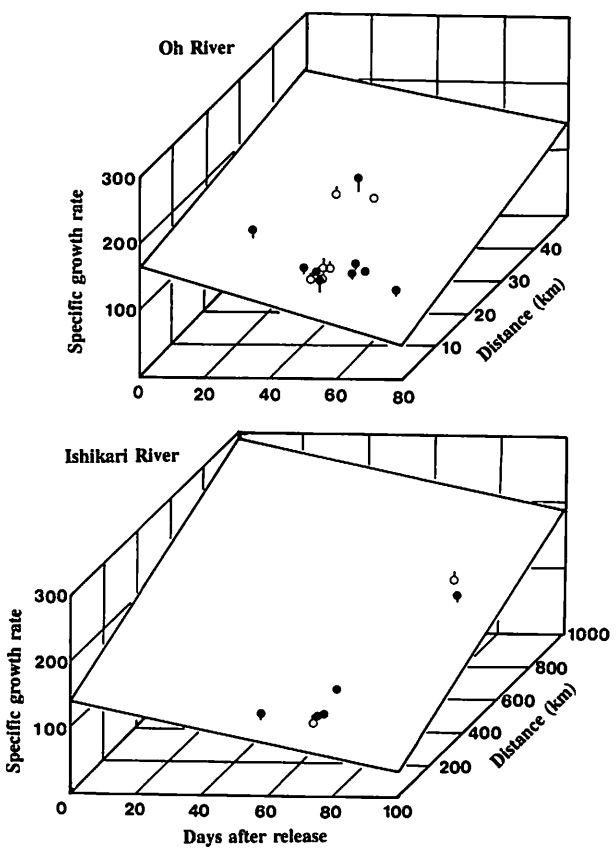

Figure 3. Multiple regression analysis in specific growth rate (G) of marked juveniles on days after release (t) and distance from release river (k; km) in chum salmon populations released from the Oh and Ishikari rivers (Modified from Kaeriyama 1986). Oh River: $G = -1.450t + 1.853k + 163$, Ishikari River: $G = -1.010t + 0.147k + 140$.

than others. Juveniles caught offshore preyed on pelagic organisms such as *Themisto japonica* and *Euphausia pacifica*. They showed much better growth than those

from neritic or inshore waters. The latter juveniles fed on coastal zooplankton and terrestrial insects, respectively. On the other hand, a significant multiple regression plane reveals data for specific growth rate of marked juvenile chum salmon as a function of days after release and distance from the released river (Figure 3).

These results indicate that larger juveniles which have higher growth rate migrate more rapidly and farther away from the released river than others with increase in a population density.

IDEAL FREE DISTRIBUTION MODEL

Based on the above consideration, life histories of sockeye and chum salmon are a conditional strategy. These species take residence or migration tactics by condition of available resources. This strategy conforms to the "ideal free distribution model", modified from Fretwell and Lucas (1970).

Given in Figure 4, the X-axis is "density" of a population, the Y-axis is "growth" or "resources" of individual, and "H" is a "habitat area". The habitat area enlarges from "H1" to "H3" such as a river, coastal waters, and the ocean. For instance, juveniles stay on "H1" and keep high growth rate (more than "g1") at "low density". With increase in population density, their growth rate may decrease. When the density exceeds "d1" and the growth rate falls less than "g1", juveniles migrate to wider habitats such as "H2" and "H3". Even if the density is low, fish which have higher growth rate or more resources ("g3") can migrate to wider habitat ("H2" or "H3").

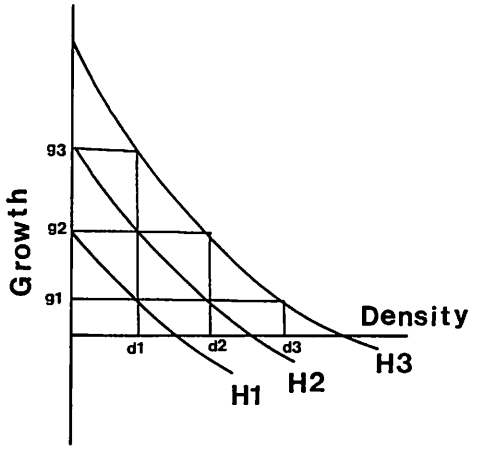

Figure 4. Ideal free distribution model of juvenile *Oncorhynchus* (description in text).

INDIVIDUAL GROWTH REDUCTION REGARDING THE POPULATION-DENSITY-DEPENDENT EFFECT

The intraspecific competition leads to a population-density-dependent effect such as changes in individual growth and age composition of a population.

A decrease in mean fork length of age 3–5 Japanese chum salmon was significant after the late 1970s when the population began to increase exponentially. In the Ishikari River, the mean fork length of age 3–5 female chum salmon showed a significant decreasing trend from 1979 to 1984 and has leveled off since 1985 (Figure 5A). The average fork length of age 4 female declined by about 9% from the late 1970s (687 mm) to the early 1980s (638 mm). A significant negative relationship between chum salmon population size in Hokkaido and their fork length was observed ($r < -0.8$, $P < 0.001$; Figure 5B). Age composition of a brood year population has changed with recent large returns of Hokkaido chum salmon. The average age for returning adults was about 3.7 years until the 1972 brood year, but it has increased gradually and attained over 4 years since the 1980 brood year (Figure 6A). A significant positive relationship between population size and average age of a population at maturity was observed ($r = 0.909$, $P < 0.001$; Figure 6B). In this way, the observed decrease

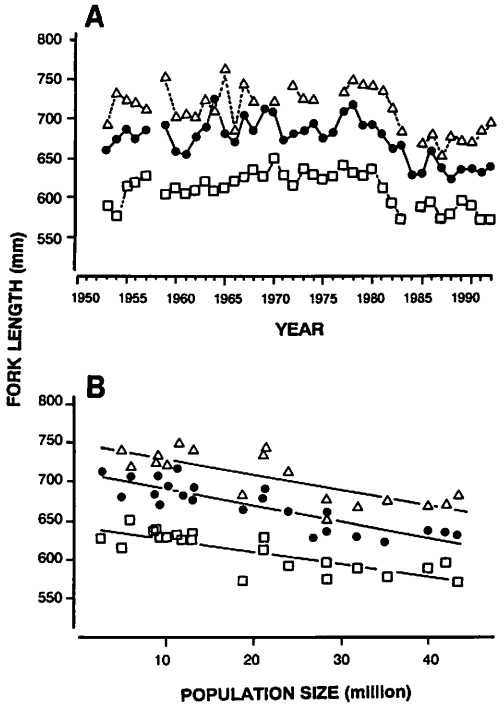

Figure 5. Annual change in fork length of female adult chum salmon (A), and relationship between Hokkaido population size and fork length of age 3–5 female adult chum salmon returning to the Ishikari River in 1953–1992 (B). Age-3 (□): $r = -0.803^{***}$, Age-4 (●): $r = -0.869^{***}$, Age-5 (△): $r = -0.801^{***}$.

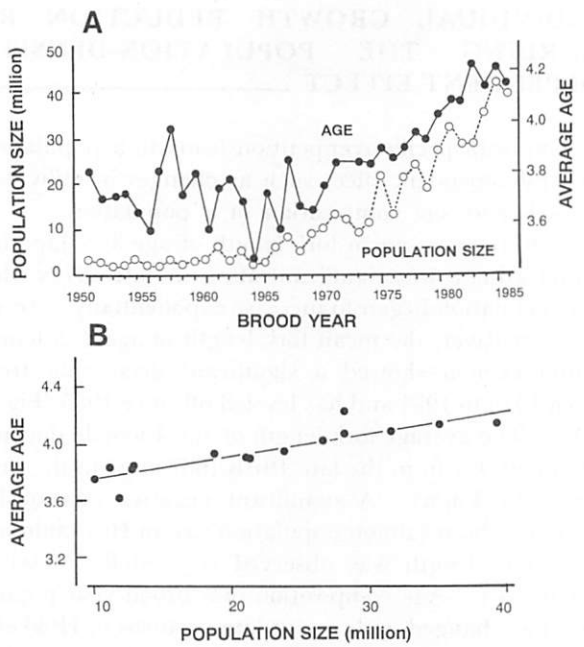

Figure 6. Annual changes in number and average age of adult chum salmon during the brood years of 1950–1986 (A), and relationship between number (P) and average age (A) at maturity of adult chum salmon during the brood years of 1971 to 1986 returning to Hokkaido (B). $A = 0.0151P + 3.6097$ ($r = 0.9088$***).

in body size has been synchronous with the increase in age at maturity.

Figure 7 demonstrates normal and narrow types of scales from age 4 chum salmon returning to Hokkaido in 1993. Scale width in the 3rd-year zone of narrow type is evidently smaller than those in other zones. Recently, narrow-type scales have been frequently observed in adult chum salmon returning to Japan. Annual growth in fork length of adult Ishikari River chum salmon from 1976 to 1992 is estimated by back-calculation procedures in an allometry formula between scale radius (S, mm) and fork length (L, mm): $L=260\ S^{0.839}+45$ ($r=0.932$, $P<0.001$), where a value "45" exhibits the fork length at squamation (Kaeriyama 1986; Table 1). Results from Table 1 are as follows:

1) Chum salmon grew over 50% of adult size in the first year of oceanic life,
2) Their growth in the first year of oceanic life did not show a significant difference between age 3, 4, and 5, except for age 5 from 1985 to 1992,
3) In the third to fifth years of oceanic life, annual growth of chum salmon decreased with increasing age,
4) Growth reduction in the third year of ocean life was considerably higher than those in the other years, and
5) Growth reduction in the first year of ocean life increased with increasing age.

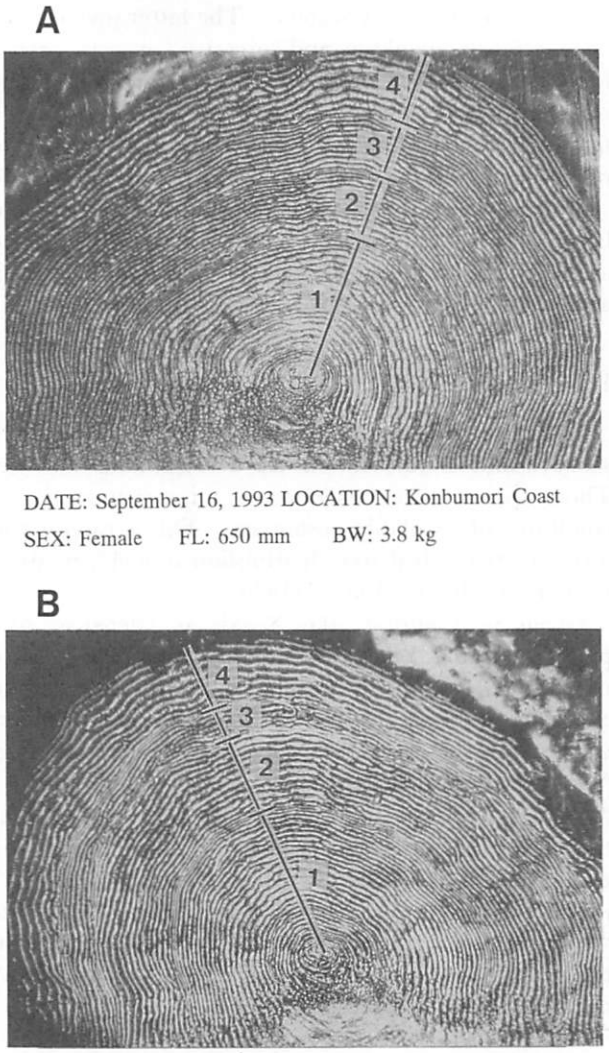

Figure 7. Photographs of normal (A) and narrow (B) types of scale from age-4 chum salmon returning to Hokkaido in 1993.

These findings suggest that the growth reduction of Japanese chum salmon occurs after the second year of oceanic life, especially in the third year when they extend their migration to the eastern North Pacific Ocean and the Bering Sea.

Ogura and Ito (1994) documented that the recent distribution area of maturing Japanese chum salmon has extended much further south and west than shown in previous studies, and concluded that extensive stock enhancement of Japanese chum salmon was associated with enlargement of the known distribution area in the North Pacific Ocean. This indicates that increase in population size of Japanese chum salmon is associated with enlargement of their known distribution area as well as individual growth reduction.

Oceanic environment, selection, and heritability by

Table 1. Calculative growth reduction of adult chum salmon returning to the Ishikari River from 1976 to 1992. Annual growth is estimated from the back calculation in allometry formula between the scale radius (S, mm) and fork length (L, mm): $L=260S^{0.839}+45$.

		Annual mean growth		Growth reduction* mm (%)
		1976–1980 (mm)	1985–1992 (mm)	
Age 3	1st-year	364	359	5 (1.4)
	2nd-year	131	124	7 (5.3)
	3rd-year	116	96	20 (17.2)
	Total	611	579	32 (5.2)
Age 4	1st-year	364	351	13 (3.6)
	2nd-year	130	116	14 (10.8)
	3rd-year	102	80	22 (21.6)
	4th-year	96	82	14 (14.6)
	Total	692	629	63 (9.1)
Age 5	1st-year	367	345	22 (6.0)
	2nd-year	123	112	11 (8.9)
	3rd-year	84	70	14 (16.7)
	4th-year	75	72	3 (4.0)
	5th-year	71	71	0 (0)
	Total	720	670	50 (6.9)

* Growth reduction = Annual mean growth (1976–1980)–Annual mean growth (1985–1992).

fisheries and hatchery practices may not be the main factor affecting the recent body size changes because of favorable oceanic condition for Pacific salmon and synchronization between the decrease in body size and the increase in average age at maturity. Therefore, population-density-dependent effect appears to have a great potential for the cause of the individual growth reduction of chum salmon returning to Japan.

CONCLUSION

The biomass condition of sockeye and chum salmon populations is controlled by the population-density-dependent effect as well as by the environmental change in their habitat. In the salmon enhancement program, therefore, it is extremely important to control an optimum of population system by clarifying the mechanism of population regulation such as population-density-dependent effect, and by monitoring biological characters of population such as migration pattern, body size, age composition, and fecundity.

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LITERATURE CITED

Beamish, R. J., and D. R. Bouillion. 1993. Pacific salmon production trends in relation to climate. Can. J. Fish. Aquat. Sci. 50: 1002–1016.

Brodeur, R. D., and D. M. Ware. 1992. Long-term variability in zooplankton biomass in the subarctic Pacific Ocean. Fish. Oceanogr. 1: 32–38.

Fretwell, S. D., and H. L. Jr. Lucas. 1970. On territorial behavior and other factors influencing habitat distribution in birds. I. Theoretical development. Acta Biotheor. 19: 16–36.

Ishida, Y., S. Ito, M. Kaeriyama, S. McKinnell, and K. Nagasawa. 1993. Recent changes in age and size of chum salmon (*Oncorhynchus keta*) in the North Pacific Ocean and possible causes. Can. J. Fish. Aquat. Sci. 50: 290–295.

Kaeriyama, M. 1986. Ecological study on early life of the chum salmon, *Oncorhynchus keta* (Walbaum). Sci. Rep. Hokkaido Salmon Hatchery (40): 31–92. (In Japanese with English summary).

Kaeriyama, M. 1989. Aspects of salmon ranching in Japan. Physiol. Ecol. Jpn. Spec. Vol. 1: 625–638.

Kaeriyama, M. 1991. Dynamics of the lacustrine sockeye salmon population in Lake Shikotsu, Hokkaido. Sci. Rep. Hokkaido Salmon Hatchery (45): 1–24. (In Japanese with English summary).

Ogura, M., and S. Ito. 1994. Change in the known ocean distribution of Japanese chum salmon, *Oncorhynchus keta*, in relation to the progress of stock enhancement. Can. J. Fish. Aquat. Sci. 51: 501–505.

Pearcy, W. G. 1992. Ocean ecology of North Pacific salmonids. Univ. Washington Press, Seattle, 177 pp.

Rogers, D. E., and G. T. Ruggerone. 1993. Factors affecting marine growth of Bristol Bay sockeye salmon. Fish. Res. 18: 89–103.

Thomas, G. L., and O. A. Mathisen. 1993. Biological interactions of natural and enhanced stocks of salmon in Alaska. Fish. Res. 18: 1–17.

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DOWNSTREAM MIGRATORY BEHAVIORS AND ENDOCRINE CONTROL OF SALMONID FISHES

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ABSTRACT

Downstream migratory behavior and its hormonal control of salmonid studies show that masu salmon yearlings were territorial during the parr and presmolt stages, less aggressive during parr-smolt transformation, but highly aggressive in post-smolt stage. Subordinate fish had high levels of plasma cortisol, but the cortisol levels of dominants were as low as those of control fish in the stock tank. Thyroxin (T_4) treated presmolts showed less aggressive behavior than control fish. Before the migratory period, chum salmon fry and coho salmon yearlings preferred shaded areas to open water. Triiodothyronine (T_3) or T_4 treatment induced a preference for open water. During the premigratory season, chum salmon fry swam against the flow of current. The T_3 treatment changed their swimming direction to downstream. Underyearling sockeye salmon showed a natural surge of T_4 two weeks before downstream migration occurred. The same group of fish reared in artificial streams were induced to migrate downstream following T_3 and T_4 treatment. Underyearling coho salmon showed greater preference for sea water or undirected searching behavior after treatment with growth hormone, T_4 and cortisol. These findings suggest that thyroid hormones, growth hormone, and cortisol play an important role not only in smolting but also in the behaviors favoring downstream migration of salmonids.

INTRODUCTION

Many salmonids are anadromous and migrate down to the ocean after morphological, physiological, and behavioral changes; these changes are termed parr-smolt transformation, smoltification or smolting (reviewed by Folmar and Dickhoff 1980; Hoar 1988; Boeuf 1994). Timing of smolting and downstream migration vary in species, pink salmon *Oncorhynchns gorbuscha* and chum salmon *O. keta* are able to enter sea water soon after emergence. No chum salmon fry remain in the river for more than a few days after stocking (Iwata and Komatsu 1984). Sockeye salmon acquire morphological change of silvery body color in their first late spring and swim down to the nursery lakes. In contrast, masu salmon *O. masou masou*, coho salmon *O. kisutch*, Atlantic salmon *Salmo salar* and other species spend one or more years in freshwater habitats; some of them migrate down to the sea and others stay in streams their whole life.

Aggressive behaviors of salmonid species were observed easily in a tank with a few fish showing light body color and the rest of the fish with dark color (Keenleyside and Yamamoto 1962). Dominance hierarchies increase stress in subordinate individuals. The highest level of interrenal activity occurs in the lowest ranked rainbow trout in a dominance hierarchy (Noakes and Leatherland 1977). The subordinates show markedly increased cortisol level in the blood (Laidley and Leatherland 1988).

The hormonal controls of the parr-smolt transformation in salmonids have been studied, relating to morphological transformation, osmoregulation, growth, and many other aspects (Bern and Nishioka 1993; Boeuf 1994). Hoar (1939) reported the dramatic elevation in thyroid function accompanying smolting of Atlantic salmon. Circulating thyroxin concentrations were found to increase dramatically during smolting of coho salmon (Dickhoff et al. 1978). In this paper, I summarize our recent studies in several behaviors of Pacific salmon juveniles in fresh water which occur in migratory fish, and describe the effects of thyroid hormones and other hormones on the territorial and downstream behaviors and seawater preference; these results suggest a research direction in mechanisms controlling the migration of salmonid juveniles.

1. TERRITORIAL BEHAVIOR

Comparison among species

It is generally observed that river resident salmonids form territories during the spring-autumn growing season (Grant and Noakes 1987; Grant 1990). Pink

and chum salmon migrate down river soon after emergence, some still bearing yolk (Hoar 1951; Houston 1957; McInerney 1964). Sockeye fingerlings after a few months also leave the river habitat to move into lakes. Although chum fry have been observed to display aggressive behavior and form a dominance hierarchy with prolonged rearing in fresh water (Yamagishi et al. 1981), these three species are less territorial in the river, because they migrate downstream before the increase in food requirement or they dependless on food in the river. Salmons such as masu and coho do not smoltify in their first spring in streams, although they are active in feeding and growth. These fish and other yearling or older migratory species compete for food during riverine life, because the fish do not acquire sufficient seawater adaptability for migration to the ocean. If food requirement is much larger than the supply, then protective aggression for feeding territories is necessary for guaranteeing growth and survival.

In our recent observation during late spring and summer, underyearlings of non-anadromous brook trout *Salvelinus fontinalis*, show the highest level of aggressive behavior, followed by masu salmon, rainbow and steelhead trout (both *O. mykiss*). On the other hand, amago, *O. masou ishikawae*, and coho are moderately aggressive, and kokanee and sockeye (both *O. nerka*) and chum salmon are the least aggressive species. These results indicate that species inhabiting for longer duration in a river are aggressive, while the least aggressive species are earlier migrators that have stable schooling behaviors. Many years ago, Rounsefell (1958) attempted to evaluate the degree of anadromy of various salmonids by several different characteristics associated with migration and the anadromous habit. His results show four groups from the most extreme of the obligatory anadromous forms, represented by pink and chum salmon, to the wholly freshwater Arctic char *S. namaychush*.. Sockeye, pink and chum salmon school actively and tend to live a pelagic life; the other species are territorial, occasionally forming loose aggregates. This separation, on the basis of social behavior, is fully in agreement with divisions based on taxonomic characteristics (Hoar 1976). Aggressiveness of salmonids, which we observed, seems to coincide with their dependency on river habitats.

Changes in aggression according to smoltification

Territorial aggressiveness decreases with the increase in anadromy of species. Within a species, migratory masu yearlings show a gradual decrease in aggressive behavior as smolting progresses, and reach the minimum level of aggression at the peak of smolting. Blood T_4 level increased with development of smolting in masu salmon (Hutchison and Iwata, in prep.). Large peaks in downstream movement of masu and Biwa salmon *O. masou* subsp., occurred several times in a season and coincided with rainfall and sharp increase of plasma T_4 concentration (Yamauchi et al. 1985; Fujioka et al. 1990). The T_4 and T_3 treatments clearly decreased the aggressive behavior of presmolt masu salmon; these data confirm findings in Pacific and Atlantic salmon (Hoar 1951; Godin et al. 1974). Our data suggest that a territorial phase shifts to a schooling phase during smolting and thyroid hormones stimulate this process. In future studies the reduction of aggressive behavior is considered a key factor not only to lead the fish to school formation but also to allow the onset of downstream migration.

Territorial stress and cortisol

Cortisol has been known as a hormone that promotes seawater adaptability (Specker 1982). Also the high level of cortisol by transport stress reduces the relative fitness of stressed fish in the wilds, resulting in low survival rates to adulthood (Schreck et al., 1989). When we placed a group of masu salmon parr and smolts within a confined space of tank, dominant parr show no increase in plasma cortisol level, however, subordinates have high levels of cortisol concentration. These subordinates stay in a corner of the tank and remain inactive even if they are exposed to nip of dominant fish, however, the subordinate fish maintain similar seawater adaptability such as smolts in the stock tank. It is necessary to compare the behaviors and their physiological responses in the wilds with in a limited enclosed space under experimental conditions, because usually we observe aggregating smolts with dark body color at the lower part of a pool where a dominant parr (precociously matured masu salmon, for example) occupies most of the space in the pool.

2. CHANGES IN PHOTOTAXIS

In preparation for downstream migration of salmonids, the fish abandon its habitat to encounter a migratory school. Chum alevins or fry on the resident phase inhabit in a shade by water-weeds, rocks, or at the bottom layer of a hollow in the river bed and swim out for feeding and back to the shade (Iwata 1982). The negative phototaxis, preference for shade or to be in shelter, is a common behavior of river-resident salmonids (Hoar 1951). Although major migration occurs during the night in Atlantic salmon (Thorpe 1982) and others (Hoar 1951), chum salmon migrators form schools in open water during the daytime at the peak period of seaward migration (Iwata and Komatsu 1984). In our previous study (Iwata et al. 1989), chum fry changed their preference from shade to open water after T_4 treatment, although thiourea treatment had no effect. Coho smolts immersed in T_3 for 3 days also

preferred open water to the shaded area; the effect remained for a week after treatment. Thyroid hormones cause the fish to move to open water in daytime where it may be advantageous for the fish to form schools and migrate seaward.

3. SEAWATER ADAPTABILITY AND SALINITY PREFERENCE

The completion of smolting includes the development of salinity tolerance allowing smolts to enter the ocean (see review of Hoar 1988). The capacity of seawater adaptation can be monitored by measuring plasma electrolytes (sodium and chloride) concentration after seawater entry (e.g. Clarke et al. 1989), and gill Na_+-K_+ATPase activity (e.g. McCormick and Bern 1989). Plasma growth hormone (GH) and liver and gill insulin-like growth factor I (IGF-I) mRNA increase in freshwater fish during smoltification (Duguay et al. 1994). The hypoosmoregulatory ability is stimulated by GH (Bolton et al. 1987). Pituitary GH secretion, plasma GH level and its turnover rates increase after transfer to seawater (Sakamoto et al. 1991); GH also causes production of IGF-I in the liver, gill and kidney, and sensitizes interrenal to adrenocorticotropin (ACTH), increasing cortisol secretion; cortisol, GH and IGF-I may be involved in stimulating seawater adaptation (Sakamoto and Hirano 1993; Sakamoto et al. 1993). The interaction between thyroid hormones, cortisol, GH and IGF-I should be studied with relation to their migratory behaviors.

Temporal changes in salinity preference have been reported for several species of Pacific salmon (Houston 1957; Baggerman, 1960; Iwata et al. 1990). In pink and chum salmons that migrate to the sea shortly after hatching, seawater preference increases during the migratory period and decreases rapidly thereafter (Baggerman 1960; Iwata et al. 1986) when their seawater adaptability is depressed (Kojima et al. 1993). In a salinity preference tank constructed with two chambers including fresh water or seawater, we observed restlessness and individual searching behavior of chum fry along the Plexiglas partition wall, and the rest of fry in a group tended to follow an active searcher (Iwata et al. 1986). These exploration and following behaviors are generally observed in chum fry at the peak period of downstream migration. Exploratory behaviors and the following behavior cause the migrants to leave their habitats and allow them to form a larger school, presumably in preparation for seaward migration. Underyearling coho salmon immersed in T_4 showed a quick preference for saline water; the combination of T_4 with GH and GH alone induced restlessness and searching behavior (Iwata et al. 1990).

4. CHANGES IN RHEOTAXIS

When the freshwater residents inhabit stream, fish must swim as fast as the water flow. When smolts migrate downstream, the fish could passively drift downstream, actively swim downstream headfirst, or actively swim upstream more slowly than the river flow and thus be carried downstream tail-first (Arnold 1974).

Chum fry behavior was tested in a circulating current tank constructed with deep water and shallow passage during their migratory season. The T_3-treated chum fry appeared in the shallow passage more than the control group, whereas thyroid depressant propylthiouracil (PTU) reduced occupancy of the shallow section (Sato and Iwata, unpublished). In the shallow passage, most T_3-treated chum fry actively swam down headfirst, but PTU-treated fry did not exhibit downstream orientation. After the fish begin downstream rheotaxis, they continue swimming around the circular passage during day and night, if the experimental conditions are not changed. These results support the hypothesis that thyroid hormones are involved in initiation of downstream migratory behavior of chum fry.

5. DOWNSTREAM MIGRATORY BEHAVIOR

Smolting is a necessary development for successful residence in the marine environment, although apparent smolts do not always migrate to the sea. For example in precociously maturing masu salmon in presmolt stage, testosterone is considered to inhibit completion of smoltification (Ikuta et al. 1985); they become desmolts after migratory season. During smolting, we can recognize several stages of the process by distinct features such as parr marks, body color, black pigmentation at outer edges of fins, and body shape, although not all fish exihibiting these characteristics must go to sea (Gorbman et al. 1982). Also it is possible to recognize smolting masu salmon that do not migrate down stream by incompletion of smolting (Kubo 1974). Some masu salmon smolts do not join migrating schools during the restlessness phase in the river. These fish may be injured by nipping, infected with disease or parasites. It is also possible that schooling smolts have not reached sufficient density or there may be other reasons that switches for downstream migration have not been activated. From the behavioral observations mentioned above, we assume that the downstream migration of salmonids is decided positively by the fish, and thyroid function may be involved in this mechanism.

The major downstream movement occurs at dusk and in the night, as many researchers have reported in various species (Hoar 1951; Thorpe 1982). We

examined the effects of illumination intensity on the nearest neighbor distance (NND) of coho salmon in an experimental aquarium (Azuma and Iwata 1994). The NNDs in intact fish were divided into three groups according to the illumination intensities, and the dusk condition at 0.01 to 0.4 lux induced larger NND than daytime condition (4–4000 lux). The schooling behavior at dusk in the trough and the experimental aquarium is not random swimming as would be seen with blinded fish or fish in complete darkness. Feeding T_4 or T_3 supplemented diet increased plasma hormone levels several hours after administration. The downstream migration of the treated fish occurred during and after dusk between 1800 and 2300. Treated animals migrated more than control fish (Iwata and Tagawa, unpublished data 1988–1993). However, in a repeat 1994 experiment in sockeye salmon with continuous oral-administration of T_4 and thyroid depressant thiourea (TU) during migratory season showed an opposite results; the TU-treated fish swam down the trough continuously for two months, followed by the control group, while the fewest migrators were fish treated with T_4 every 2 weeks (Iwata et al. unpublished data). Birks et al. (1985) demonstrated similar effects with T_4 injection and determined that the treatment inhibited the downstream progress of steelhead trout *Oncorhynchus mykiss* in a model system for 2 weeks. Ambiguous effects of T_4 on downstream migration was reported for an experiment in which T_4 treated Atlantic salmon showed no qualitative difference in movement in a stream (Youngson et al. 1989). This confusion in thyroid hormone effects on the behavior of downstream migration is similar to their effects on osmoregulation of salmonids (see review of Hoar 1988).

CONCIUSION

Salmonids are characterized by distinct life style with a wide range of migration. Performance of downstream migration in salmonids is variable among not only genera or species but also populations or even in individuals of some species like masu salmon. The most clear difference between these salmonids appears that they may stay in fresh water their whole life, whereas others migrate to the sea.

The parr-smolt transformation occurs in association with the hormonal surges in the blood including insulin, thyroid hormones, GH, sex steroids, cortisol, prolactin, catecholamines, and many others (see reviews by Hoar 1988; Boeuf 1994). We expect further research in various modes of hormonal action, receptors, neuropeptide hormones, and neuroelectric activities that regulate switching mechanism of the behaviors. It is fairly reasonable to say that thyroid function at the peak of smolting results in T_4 secretion in a response to environmental cues. From an ecological viewpoint, the importance of the T_4 surge is that most of individuals in a river recognize the cue, whether or not T_4 eventually stimulates a part of a serial process of behaviors in downstream migration. In this paper evidence is presented that the thyroid functions are considered to be involved in the determination of smolt specific behaviors such as schooling, phototaxis and rheotaxis; thyroid hormones may be secondarily effective on downstream migratory movement of salmonids. Moreover, it is important to say that the potential role of thyroxin in olfactory learning and imprinting in migrating smolts is necessary, because a correlation between thyroid activity and long-term olfactory memory in Atlantic salmon had been reported (Morin et al. 1989). Recently, we reported that cells producing gonadotropin releasing hormone (GnRH) developed actively during the peak period of downstream migratory period of chum salmon; thyroid hormone increase, GnRH gene expression and onset of migratory behavior occurred coincidentally (Parhar et al. 1994,1995; Parhar and Iwata unpablished). Hypothalamic control is also necessary to involve in the study.

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LITERATURE CITED

Arnold, G. P. 1974. Rheotropism in fishes. Biol. Rev. 49: 515–576.

Azuma, T. and M. Iwata 1994. Influences of illumination intensity on the nearest neighbor distance in coho salmon *Oncorhynchus kisutch*. J. Fish Biol. 45: 1113–1118.

Baggerman, B. 1960. Salinity preference, thyroid activity and the seaward migration of four species of Pacific salmon (*Oncorhynchus*.). J. Fish. Res. Board Can. 17: 295–322.

Bern, H. A. and R. S. Nishioka 1993. Aspects of salmonid endocrinology: the known and the unknown. Bull. Fac. Fish., Hokkaido Univ. 44: 55–67.

Birks, E. K., R. D. Ewing, and A. R. Hemmingsen 1985. Migration tendency in juvenile steelhead trout, *Salmo gairdneri* Richardson, injected with thyroxine and thiourea. J. Fish Biol. 26: 291–300.

Boeuf, G. 1994. Salmonid smolting: a pre-adaptation to the oceanic environment. In: G. C. Rankin and G. B. Jensen (Eds), Fish Ecophysiology, pp. 105–135. Capman & Hall, London.

Bolton, J. P., N. L. Collie, H. Kawauchi, and T. Hirano 1987. Osmoregulatory actions of growth hormone in rainbow trout (*Salmo gairdneri*). J. Endocrinol. 112: 63–68.

Clarke, W. C., J. E. Shelbourn, T. Ogasawara, and T. Hirano 1989. Effects of initial daylength on growth, seawater adaptability and plasma growth hormone levels in underyearling coho, chinook and chum salmon. Aquaculture, 82: 51–62.

Dickhoff, W. W., L. C. Folmar, and A. Gorbman 1978. Changes in

plasma thyroxine during smoltification of coho salmon, *Oncorhynchus kisutch*.. Gen. Comp. Endocrinol. 36: 229–232.

Duguay, S. J., P. Swanson, and W. W. Dickhoff 1994. Differential expression and hormonal regulation of alternatively spliced IGF-I mRNA transcripts in salmon. J. Molec. Endocrinol. 12: 25–37.

Folmar, L. C. and W. W. Dickhoff 1980. The parr-smolt transformation (smoltification) and seawater adaptation in salmonids. A review of selected literature. Aquaculture 21: 1–37.

Fujioka, Y., S. Fushiki, M. Tagawa, T. Ogasawara, and T. Hirano 1990. Downstream migratory behavior and plasma thyroxine levels of Biwa salmon *Oncorhynchus rhodurus*. Nippon Suisan Gakkaishi 56: 1773–1779.

Godin, J. G., P. A. Dill, and D. E. Drury 1974. Effects of thyroid hormones on behavior of yearling Atlantic salmon (*Salmo salar*). J. Fish. Res. Board Can. 31: 1787–1790.

Gorbman, A., W. W. Dickhoff, J. L. Mighell, E. F. Prentice, and F. W. Waknitz 1982. Morphological indices of developmental progress in the parr-smolt coho salmon. Aquaculture 28: 1–19.

Grant, J. W. A. 1990. Aggressiveness and the foraging behavior of young-of-the-year brook charr *Salvelinus fontinalis*. Can. J. Fish. Aquat. Sci. 47: 915–920.

Grant, J. W. A. and D. L. G. Noakes 1987. Simple model of optimal territory size for drift-feeding fish. Can. J. Zool. 65: 270–276.

Hoar, W. S. 1939. The thyroid gland of the Atlantic salmon. J. Morphor. 65: 257–259.

Hoar, W. S. 1951. The behaviour of chum, pink and coho salmon in relation to their seaward migration. J. Fish. Res. Board Can. 8: 241–263.

Hoar, W. S. 1976. Smolt transformation: evolution, behavior, and physiology. J. Fish. res. Board Can. 33: 1232–1252.

Hoar, W. S. 1988. The physiology of smolting salmonids. In W. S. Hoar and D. J. Randall Eds), Fish Physiology, Vol. 11B, pp. 273–323. Academic Press, New York, NY.

Houston, A. H. 1957. Responses of juvenile chum, pink and coho salmon to sharp seawater gradients. Can. J. Zool. 35: 371–383.

Ikuta, K., K. Aida, N. Okumoto, and I. Hanyu 1985. Effects of thyroxine and methyltestosterone on smoltification of masu salmon (*Oncorhynchus masou*). Aquaculture 45: 289–304.

Iwata, M. 1982. Transition of chum salmon fry into salt water. Proc. Salmon and Trout Migratory Behavior Symposium. Univ. Washington, pp. 204–207.

Iwata, M. and S. Komatsu 1984. Importance of estuarine residence for adaptation of chum salmon (*Oncorhynchus keta*) fry to seawater. Can. J. Fish. Aquat. Sci. 41: 744–749.

Iwata, M., H. Ogura S. Komatsu, and K. Suzuki 1986. Loss of seawater preference in chum salmon (*Oncorhynchus keta*) fry retained in fresh water after migration season. J. Exp. Zool. 240: 369–376.

Iwata, M., T. Yamanome, M. Tagawa, H. Ida, and T. Hirano 1989. Effects of thyroid hormones on phototaxis of chum and coho salmon juveniles. Aquaculture 82: 329–338.

Iwata, M., K. Yamauchi, R. S. Nishoka, R. Lin, and H. A. Bern 1990. Effects of thyroxine, growth hormone and cortisol on salinity preference of juvenile coho salmon (*Oncorhynchus kisutch*). Mar. Behav. Physiol. 17: 191–201.

Keenleyside, M. H. and F. T. Yamamoto 1962. Territorial behaviour of juvenile Atlantic salmon (*Salmo salar* L.). Behaviour 19: 1539–169.

Kojima, H., M. Iwata, and T. Kurokawa 1993. Development and temporal decrease in seawater adaptability during early growth in chum salmon, *Oncorhynchus keta*. Aquaculture 118: 141–150.

Kubo, T. 1974. Notes on the phase diferentiation and smolt transformation of juvenile masu salmon (*Oncorhynchus masou*). Sci. Rep. Hokkaido Salmon Hatchery, 28: 9–26. (in Japanese with English summary)

Laidley, C. W. and J. F. Leatherland 1988. Cohort sampling anesthesia and stocking density effects on plasma cortisol thyroid hormone metabolite and ion levels in rainbow trout *Salmo gairdneri* Richardson. J. Fish Biol. 33: 73–88.

McCormick, S. D. and H. A. Bern 1989. In vitro stimulation of Na^+-K^+-ATPase activity and ouabain binding by cortisol in coho salmon gill. Am.J.Physiol. 256: R707–R715.

McInerney, J. E. 1964. Salinity preference: an orientation mechanism in salmon migration. J. Fish. Res. Board Can. 21: 995–1018.

Morin, P. P., J. J. Dodson, and F. Y. Dore 1989. Thyroid activity concomitant with olfactory learning and heart rate changes in Atlantic slamon *Salmo salar* during smoltification. Can. J. Fish Aquat. Sci. 46: 131–136.

Noakes, D. L. G. and J. F. Leatherland 1977. Social dominance and interrenal cell activity in rainbow trout, *Salmo gairdneri* (Pisces, *Salmonidae*). Environ. Biol. Fishes, 2: 131–136.

Parhar, I. S., Koibuchi, N., Sakai, M., Iwata, M. and Yamaoka, S., 1994. Gonadotropin-releasing hormone (GnRH): expression during salmon migration. Neurosci. Letters 172: 15–18.

Parhar, I. S., M. Iwata, D. W. Pfaff, and M. Schwanzel-Fukuda 1995. Enbryonic development of gonadotropin-releasing hormone neurons in the sockeye salmon. J. Comp. Neurol. 362: 2–16.

Rounsefell, G. A., 1958. Anadromy in North American Salmonidae. U.S. Fish. Wildl. Serv. Fish. Bull. 58: 171–185.

Sakamoto, T. and T. Hirano 1993. Expression of insulin-like growth factor I gene in osmoregulatory organs during seawater adaptation of the salmonid fish: possible mode of osmoregulatory action of growth hormone. Proc. Natl. Acad. Sci. USA 90: 1912–1916.

Sakamoto, T., M. Iwata, and T. Hirano 1991. Kinetic studies of growth hormone and prolactin during adaptation of coho salmon, *Oncorhynchus kisutch*, to different salinities. Gen. Comp. Endocrinol. 82: 184–191.

Sakamoto, T., S. D. McCormick, and T. Hirano 1993. Osmoregulatory actions of growth hormone and its mode of action in salmonids: a review. Fish Physiol. Biochem. 11: 1–6.

Schreck, C. B., M. F. Solazzi, S. L. Johnson, and T.E.Nickelson 1989. Transportation stress affects performance of coho salmon, *Oncorhynchus kisutch*. Aquaculture 82: 15–20.

Specker, J. L. 1982. Interrenal function and smoltification. Aquaculture 28: 59–66.

Thorpe, J. E. 1982. Migration in salmonids with special reference to juvenile movements in fresh water. In E. L. Brannon and E. O. Salo (Eds), Proc. Salmon and Trout Migratory Behavior Symposium, pp. 86–97. Univ. Washington, Seattle, WA.

Yamagishi, H., T. Matsushima, and M. Nakamura 1981. Note on the aggressive behaviour of juvenile chum salmon *Oncorhynchus keta* (Walbaum). Sci. Rep. Hokkaido Salmon Hatchery 35: 25–31.

Yamauchi, K., M. Ban, N. Kasahara, T. Izumi, H. Kojima, and T. Harako 1985. Physiological and behavioral changes occurring during smoltification in the masu salmon, *Oncorhynchus masou*. Aquaculture 45: 227–236.

Youngson, A. F., L. P. Hansen, B. Jonsson, and T. F. Naesje 1989. Effects of exogenous thyroxine or prior exposure to raised waterflow on the downstream movement of hatchery-reared Atlantic salmon smolts. J. Fish Biol. 34: 791–797.

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EFFECTS OF STEROID HORMONES ON MIGRATION OF SALMONID FISHES

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ABSTRACT

The effects of gonadal steroid hormones such as androgen and estrogen on migratory behavior of salmonid fishes were studied. Precocious male juveniles of salmonids do not show parr-smolt transformation nor down-stream migration, and this process of inhibition was induced by the administration of sex steroids such as testosterone (T), 11-ketotestosterone (11-KT) and estradiol-17β (E_2). In response to homing migration of land-locked sockeye salmon in the lake, plasma T levels showed a high peak at the time when adult fish gather at the mouth of their native river in both males and females. At the time of up-stream behavior into the river, ovulation occurred in females, and gonadotropin (GTH) and 17α20β-dihydroxy-4-pregnen-3-one (DHP) were elevated in both males and females. In the feeding territorial behavior of immature ayu *Plecoglossus altivelis*, dominant fish showed significantly higher T levels than did subordinate ones, and T treatment enhanced their aggressiveness. These results suggest that sex steroids may affect not only the reproductive process but also the migratory behavior in orienting in/to the river habitat as a reproductive site.

INTRODUCTION

Gonadal steroid hormones such as androgen and estrogen play important roles in the reproductive process, and their effects on development and maturation of the gonads have been well studied in salmonid fish (Fostier et al. 1983). Some studies have also reported that sex steroids are thought to regulate breeding behavior of fish (Liley and Stacey 1983). However, still little is known about the effects of steroids on migratory behavior of salmonids. It is well-known that anadromous species of salmonids show down-stream migration after parr-smolt transformation, and reach maturity in the ocean. However, precociously mature males neither smoltify nor show down-stream migration, and this results in their remaining in the river as sneakers (Hoar 1988). This kind of parr which inhabits the river shows territorial behavior in feeding. When the adult salmon which have migrated to the ocean mature, they start homing migration back toward their native river. Since recurrent migration is motivated for spawning purposes, the physiological mechanisms of this behavior may be deeply related to the reproductive process. Thus, these facts suggest possibilities that sex steroids control migratory behavior to orientate salmonids to river habitats as spawning sites. In the present study, the effects of sex steroids on down-stream migration, homing migration and territorial behavior were investigated in salmonid fishes.

RESULTS

EFFECTS ON DOWN-STREAM MIGRATION

Salmon juveniles show a metamorphic transformation from parr to smolt, which is called smoltification, at the time of seaward migration. During this process, fish obtain a silvery color, a thin body form, and develop seawater adaptability which are all induced by the elevation of thyroid hormone, growth hormone, and cortisol (Hoar 1988). However, these changes are inhibited in precociously mature male fish, and this results in their remaining in the river as sneakers. The inhibition of smoltification could be artificially induced by the administration of sex steroids such as 17α-methyltestosterone, testosterone, 11-ketotestosterone, and estradiol-17β in masu salmon *Oncorhynchus masou masou* (Table 1) (Ikuta et al. 1985, 1987).

Down-stream behavior of juvenile land-locked sockeye salmon *O. nerka* was observed using a raceway from June through July. Fish going down the raceway showed high plasma levels of thyroxine compared to the levels of fish which stayed in the upper pond (Figure 1). Precocious mature male fish also appeared in this species, and they never went down the raceway

Table 1. The effects of sex steroids on the smoltification of masu salmon

Steroid	Inhibition of morphological change	Inhibition of seawater adptability	Secondary sex characters	Accumulation of gonadotropin in pituitary
Methltestosterone	++	++	++	++
Testosterone	++	++	+	++
11-ketotestosterone	++	++	++	+
Estradiol-17β	++	++	−	++
Dihydrotestosterone	−	−	−	−

Table 2. Comparison of Sex Ratio, Body Length (BL), Body Weight (BW), Condition Factor (CF), and Gonadosomatic Indices (GSI) in Downstream Behavior of Land-locked Sockeye Salmon

	N			BL (cm)	BW (g)	CF	GSI (%)
Stay	Immature	♀	12	16.81 ± 0.30	53.68 ± 3.05	11.16 ± 0.15	0.29 ± 0.01
	Immature	♂	9	16.59 ± 0.52	52.53 ± 4.82 *	11.20 ± 0.22 ***	0.05 ± 0.01 ***
	Precocious	♂	9	17.48 ± 0.55	71.60 ± 7.37	12.96 ± 0.24	5.85 ± 0.39
Downstream	Immature	♀	18	17.07 ± 0.22	54.12 ± 2.24	10.77 ± 0.10	0.31 ± 0.02
	Immature	♂	12	16.88 ± 0.37	53.41 ± 3.81	10.83 ± 0.26	0.04 ± 0.01

Data; Means ± S.E., *; $P < 0.05$, ***; $P < 0.001$

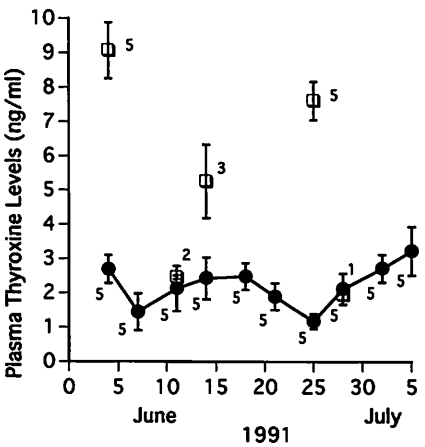

Figure 1. The changes in plasma levels of thyroxine in 1^{+}-year old land-locked sockeye salmon that showed down-stream behavior through a raceway (white squares), and that stayed in the upper tank (black circles). Vertical lines represent S.E. Small numerals represent the number of sampled fish.

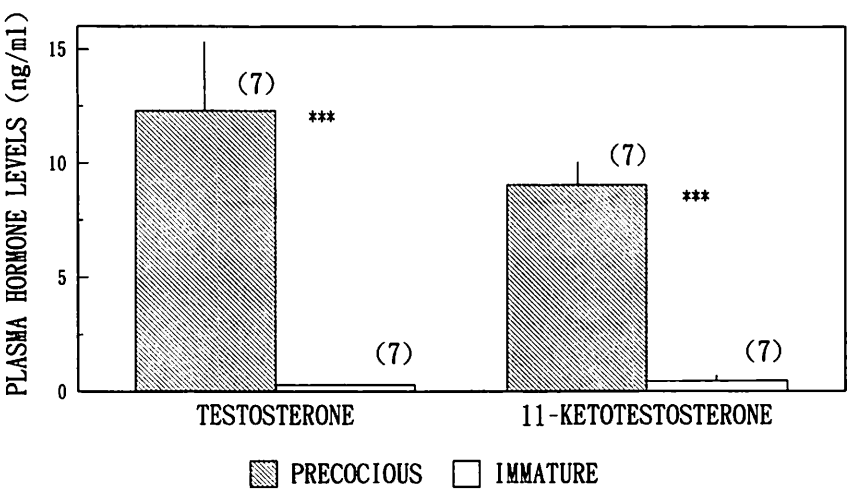

Figure 2. The plasma levels of testosterone and 11-ketotestosterone in precocious and immature male land-locked sockeye salmon. Vertical lines represent S.E. Numerals in parentheses represent the number of sampled fish. ***: $p<0.001$, student's t-test.

(Table 2). The precocious males exhibited high plasma levels of androgens such as testosterone and 11-ketotestosterone compared to the immature fish (Figure 2). These results that sex steroids inhibit not only the process of smoltification but also down-stream migratory behavior which is induced by thyroid hormone.

EFFECTS ON HOMING MIGRATION

In addition to the effects of causing fish to remain in the river, sex steroids are thought to motivate the recurrent migration of adult salmon from oceans or lakes to their native rivers. As a small-sized model for salmon migration, land-locked sockeye salmon inhabiting Lake Chuzenji were used for the analysis of hormonal changes. Ordinarily, 3-year old mature adults which have grown out in the lake return to the native stream on the premises at the Nikko Branch of the National Research Institute of Aquaculture for spawning in autumn. In examination of seasonal changes in plasma levels of reproductive hormones, testosterone showed a high peak in both male and female fish, at the

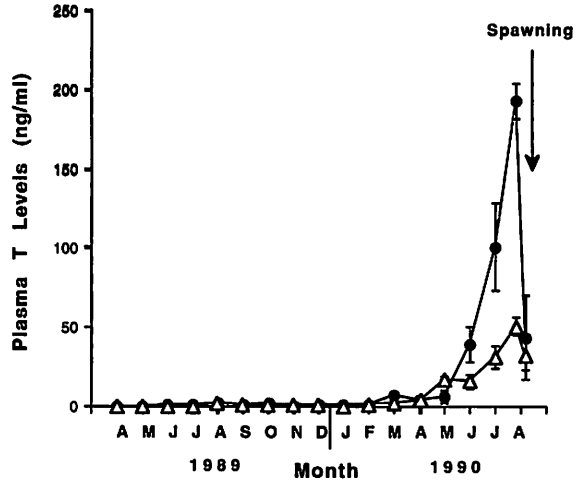

Figure 3. The changes in plasma levels of testosterone of female (black circles) and male (white triangles) land-locked sockeye salmon. Vertical lines represent S.E.

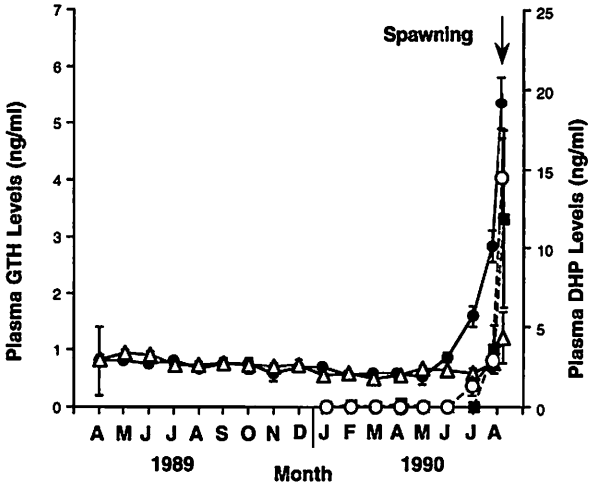

Figure 4. The changes in plasma levels of gonadotropin-II β (GTH) in female (black circles) and male (white triangles) land-locked sockeye salmon, and 17α20β-dihydroxy-4-pregnen-3-one (DHP) in female (black squares) and male (white triangles) sockeye. Vertical lines represent S.E.

moment when fish gather at the mouth of the stream (Figure 3). At the time of the final stages of migration, up-stream behavior into the river, ovulation occurred in females, and gonadotropin and 17α20β-dihydroxy-4-pregnen-3-one rapidly elevated in both males and females (Figure 4). These hormones were considered to induce final maturation of gametocytes for ovulation and spermiation. Ueda et al. (1984) and Truscott et al. (1986) reported similar changes in hormones in chum salmon *O. keta* and sockeye salmon migrating in the Pacific Ocean, respectively. These facts suggest the possibility that these hormonal changes cause a switch in the phase of a series of migratory behavior, in response to the reproductive process.

EFFECTS ON TERRITORIAL BEHAVIOR

Salmonid parr show aggressiveness and territorial behavior in defending feeding sites in their river habitats. Ayu *Plecoglossus altivelis*, similar species of salmonids, is well-known for this kind of behavior in the defense of feed algae growing on rocks (Kawanabe et al. 1957). Therefore, the ayu was used to study the effects of steroid hormones on territorial behavior. In several teleost species, mature males show aggressiveness and territorial behavior in the defense of breeding areas during the spawning season, and androgen is considered to have involvement as a stimulating factor in this behavior (Villars 1983). However, ayu shows this behavior during immature stages, in both male and female fish. Under both experimental conditions or natural river habitats, dominant fish showing the territorial behavior had significantly larger gonads (Figure 5) and higher plasma levels of testosterone (Figure 6) than did subordinate fish regardless of sex, even if the values themselves were low reflecting immaturity of the fish. Implantation of testosterone enhanced the aggressiveness and dominance of immature ayu, while estradiol-17β inhibited these behavior

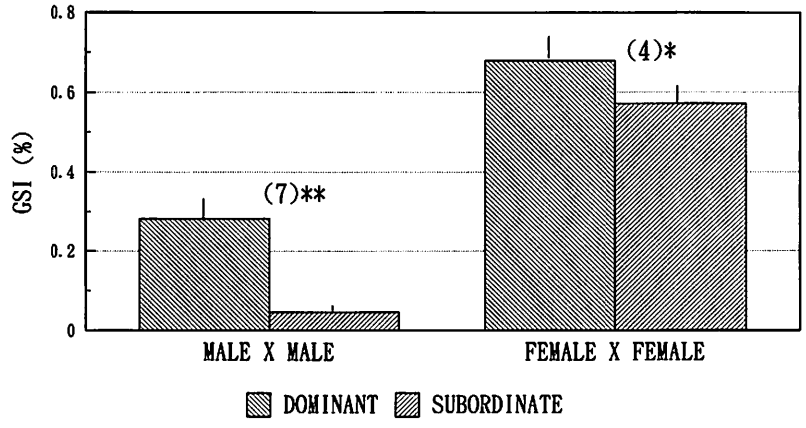

Figure 5. The gonadosomatic indices (GSI) of dominant and subordinate immature ayu in territorial behavior between pairs of same sex. Numerals in parentheses represent the number of pairs of which behavior was observed under tank conditions. *: $p < 0.05$, **: $p < 0.01$, student's t-test.

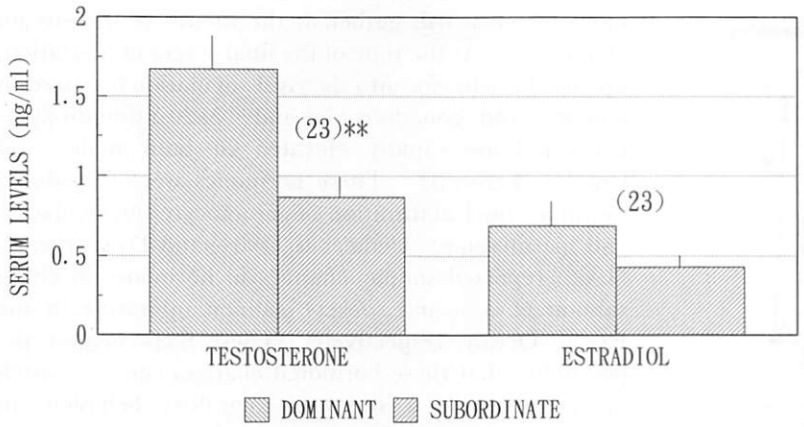

Figure 6. The serum levels of testosterone and estradiol-17β of dominant and subordinate immature ayu in territorial behavior. Numerals in parentheses represent the number of pairs of which behavior was observed under tank conditions. **: $p<0.01$, student's t-test.

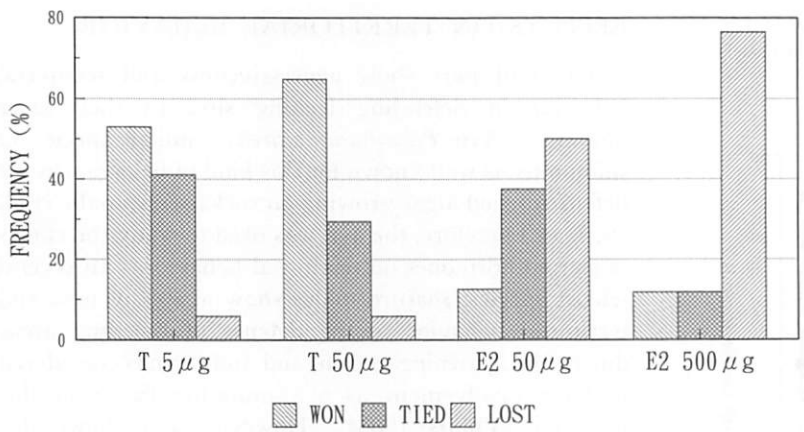

Figure 7. The outcome of territorial behavior of immature ayu in which testosterone (5 and 50 μg/fish) or estradiol-17β (5 and 50 μg/fish) were implanted. The results were estimated by frequency of fish which won, drew and lost in the fight against sham operated ayu.

(Figure 7). These results suggest that the territorial behavior is also regulated by sex steroids. Androgen may directly stimulate the tenacity of fish to their territorial areas.

CONCLUSION

These results suggest that sex steroids may play roles in not only inducing reproductive events but also in regulating factors of migratory behavior in salmonid fishes. Since the sex steroids inhibited down-stream migration, and stimulated territorial behavior in securing feeding or breeding areas, and further possibly drive homing migration toward the native river, they may effect the behavior of salmonids to orientate in/to the river habitat as a reproductive site. A recent study revealed that androgen treatment stimulated the expression of a gene for gonadotropin releasing hormone (GnRH) in brain (Amano et al. 1994). Kudo et al. (1994) reported that GnRH expression in the olfactory bulbs changed in response to homing migration in chum salmon. Therefore, there is a possibility that sex steroids affect the nervous system in brain, changing behavior and perception of environmental factors. More research is necessary in order to clarify the neurophysiological mechanisms of the effects of steroid hormones on migratory behavior in salmonid fish.

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LITERATURE CITED

Amano, M., Hyodo, S., Urano, A., Okumoto, N., Kitamura, S., Ikuta, K., Suzuki, Y., and Aida, K. 1994. Activation of salmon gonadotropin-releasing hormone synthesis by 17α-methyltestosterone administration in yearling masu salmon, *Oncorhynchus masou*. Gen. Comp. Endocrinol. 95: 374–380.

Fostier, A., Jalabert, R., Billard, B., Breton, B., and Zohar, Y. 1983. The gonadal steroids. In: Hoar, W. S., Randall, D. J., and Donaldson, E. M. (eds), Fish Physiology, Vol. IX, Part A, pp. 277–372. London, Academic Press.

Hoar, W. S. 1988. The physiology of smolting salmonids. In: Hoar, W. S., and Randall, D. J. (eds), Fish Physiology, Vol. XI, Part B, pp. 275–343. London, Academic Press.

Ikuta, K., Aida, K., Okumoto, N., and Hanyu, I. 1985. Effects of thyroxine and methyltestosterone on smoltification of masu salmon (*Oncorhynchus masou*). Aquaculture 45: 289–303.

Ikuta, K., Aida, K., Okumoto, N., and Hanyu, I. 1987. Effects of sex steroids on the smoltification of masu salmon, *Oncorhynchus masou*. Gen. Comp. Endocrinol. 65: 99–110.

Kawanabe, H. 1972. An evolutional aspect of the territoriality of ayu-fish, *Plecoglossus altivelis*, with the social structure at the southern end of its distribution. Japan. J. Ecol. 22(3): 141–149. (In Japanese; English abstract).

Kudo, H., Hyodo, S., Ueda, H., Aida, K., Urano, A., and Yamauchi, K. 1994. Involvement of gonadotropin-releasing hormone in olfactory function in salmonids. In: Kurihara, K., Suzuki, N., and Ogawa, H. (eds), Olfaction and Taste XI, pp. 761. Tokyo, Springer-Verlag.

Liley, N. R., and Stacey, N. E. 1983. Hormones, pheromones, and reproductive behavior in fish. In: Hoar, W. S., Randall, D. J., and Donaldson, E. M. (eds), Fish Physiology, Vol. IX, Part B, pp. 1–63. London, Academic Press.

Truscott, B., Idler, D. R., So, Y. P., and Walsh, J. M. 1986. Maturational steroids and gonadotropin in upstream migratory sockeye salmon. Gen. Comp. Endocrinol. 62: 99–110.

Ueda, H., Hiroi, O., Hara, A., Yamauchi, K., and Nagahama, Y. 1984. Changes in serum concentrations of steroid hormones, thyroxine and vitellogenin during spawning migration of the chum salmon, *Oncorhynchus keta*. Gen. Comp. Endocrinol. 53: 203–211.

Villars, T. A. 1983. Hormones and aggressive behavior in Teleost fishes. In Svare, B. B. (ed), Hormones and Aggressive Behavior, pp. 407–433. New York, Plenum Press.

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INTELLIGENT TAG AND ITS RECOVERY SYSTEM FOR STUDYING THE BEHAVIOR OF FREE-RANGING SALMON IN THE OCEAN

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ABSTRACT

Electric tag and its recovery system have been developed. The tag can store pressure, temperature, and serve as a fundamental statistical result of sample data. The tag is packed with satellite and/or VHF radio transmitters into the same case and is dragged by the fish. The tag comes to the sea surface after releasing from the fish at a pre-set time, and it is recovered by the ARGOS system and/or VHF direction finder. Although all devices must be smaller, the present system is considered to be useful for investigation on salmon ecology and their surroundings in the ocean.

INTRODUCTION

Radio and underwater telemetries are useful techniques for studying the behavior of marine animals. Behaviors of salmon have been examined often using both telemetries in the river, bay, and the coastal area (Fretwell 1989, Potter et al. 1992), but are seldom examined in the ocean. Ogura and Ishida (1992) examined the swimming behavior of Coho salmon (*Oncorhynchus kisutch*) using ultrasonic telemetry for about five days. Tracking the fish using ultrasonic telemetry by vessel in the ocean often had to be completed in a short time due to bad weather or exhaustion of the transmitter battery. Recently, the northern elephant seal (*Mirounga angustirostris*), Adelie penguins (*Pygoscelis adeliae*) etc. were examined without tracking the animals (Naito et al. 1990, DeLong and Stewart 1991). Scientists attached the time depth recorder (TDR) to animals at the breeding colony on land, and then released them; recaptured them on land when they returned, and collected ecological data, for example, depth, time, water temperature, light intensity, activity etc. This method surpass in collection of heterogeneous data of ecology and environments continuously for long days. So we have tried to develop the small electric tag and its recovery system for extending the application of this method to various marine animals. Although our devices are not perfected, we introduce the present results.

METHODS

INTELLIGENT TAG

The intelligent tag is a kind of micro data logger which can record the pressure and temperature, and store the fundamental statistical results of sample data for the user's discretion. The tag has four channels, although only two channels works at present (Figure 1). The data are stored in 2 M bytes of memory. Sampling can be programmed into a microprocessor unit (MPU) allowing each sensor channel to be sampled indepen-

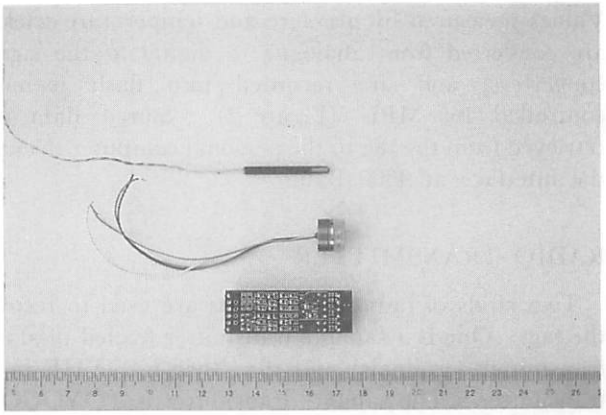

Figure 1. The sensor and electric unit for intelligent tag. Upper: temperature sensor; Middle: pressure sensor; Lower: electric circuit

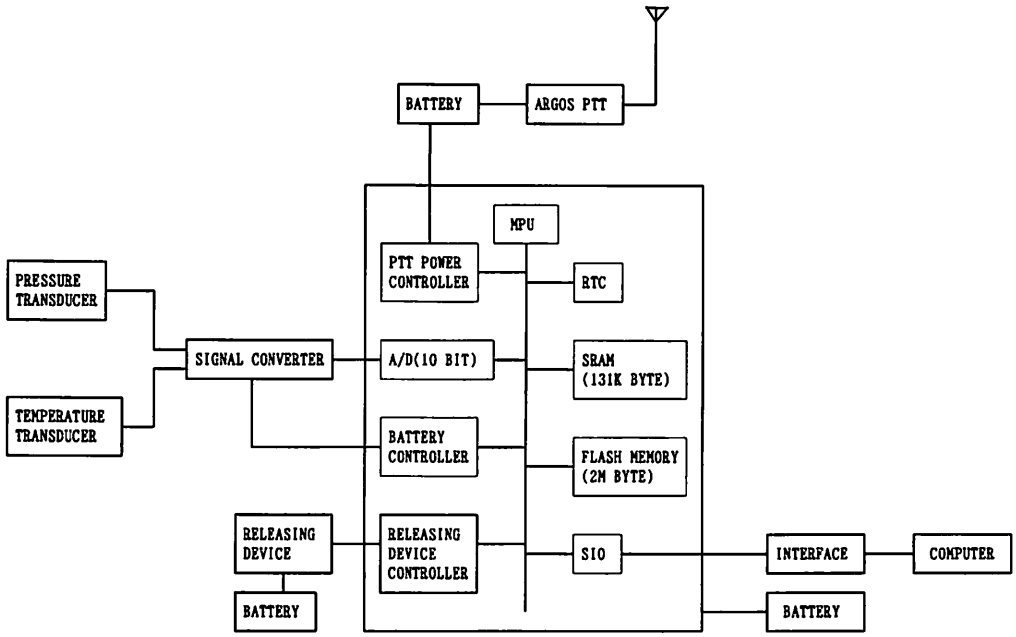

Figure 2. Block diagram of RET (recovery-type electric tag).

dently with any sequence of sampling protocols. For example, one channel can be set to take samples for 7 days at one-minute intervals, then samples taken for 10 days every three minutes, while another channel take samples every 10 seconds until exhaustion of battery.

The present tag record pressure to give depths in the range of 0–500 m $\pm$ 2 m and temperature in the range of 0–40°C $\pm$ 0.1°C. Intervals of measuring the data can be set freely within range of 1 to 1024 seconds. For example, if an interval was set at 60 seconds, the present tag can record data for up to 12 months. Calibration data for each sensor are stored in the tag so that the user can quickly download and translate the data into actual values. Even if the tag has completed its sampling regime, filled its memory, or exhausted the battery, sampling data do not disappear for using flash memory. Values measured by pressure and temperature sensors are converted from analogue to digital at the signal converters, and are recorded into flash memory controlled by MPU (Figure 2). Stored data are retrieved from the tag to the personal computer through the interface at 9600 Baud.

RADIO TRANSMITTER

Two kinds of radio transmitters are used to recover the tag. One is a satellite transmitter (called platform transmitter terminals) and the other is a VHF radio transmitter. The satellite transmitter is TOYOCOM T-2050, 401.650 MHz in carrier frequency, 0.125W in radiated output power. Size of the transmitter circuit is approximately 1.8×2.5×3.5 cm, and weight in air is about 19g including battery. Transmitter interval is 60 seconds (possible to shorten down to 40 seconds), and one lithium cell battery (ER3V) lasts 23 days. Location of PTT is detected by the ARGOS system (Taillade 1992).

VHF transmitter send the radio wave by the pulse modulation. Pulse width ranges from 10 to 15 msec and pulse repetition period is about 1.5 second. Carrier frequency is 53 MHz and radiated output power is under 3 mW. Size of transmitter is 2×2×1.5 cm, and weight in air is about 21 g including battery. One sliver oxide cell (4G13) lasts about 20 days. Transmitter is glued by epoxy resin. A four directional receiver is used to detect the source of radio wave. Maximum attainment distance of this transmitter measured on sea is about 1 km using this receiver. VHF radio transmitter and four directional receiver are manufactured by Makita Electric Laboratory Co. Ltd.

RELEASING DEVICE

This instrument releases the tag from the fish at necessary. Nylon fishing line (2 mm in diameter) is cut by heat. Release time is programmed into the MPU in the intelligent tag freely by personal computer before use (Figure 2).

PERFORMANCE

Intelligent tag and satellite transmitter are packed in the same case (recovery-type electric tag, hereafter, called

RET). RET can be found by the ARGOS system and the ARGOS direction finder (Gonio 400). The VHF radio transmitter is not packed in RET, but is attached to the outside of RET, if necessary. Although weight of prototype RET is about 500 g in air, it has little buoyancy (Figure 3).

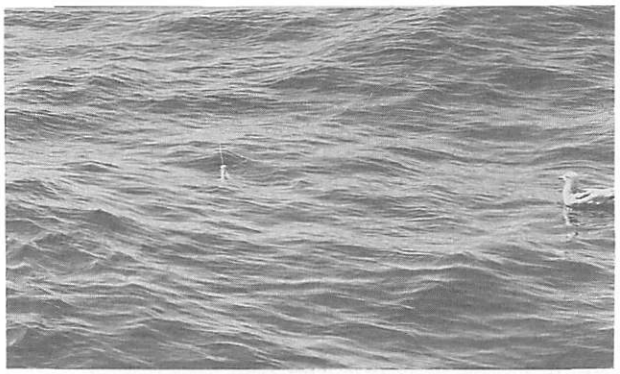

Figure 3. Prototype RET (recovery-type electric tag) drifted on the coastal area off northern Japan on April, 1995.

The releasing device is attached to the back of the dorsal fin of salmon by nylon line or sergeant string in a small water tank on ship, and then RET is knotted to it by nylon line. The water tank is placed into the sea and the salmon are released.

RET comes to the sea surface at a pre-set time and begin to transmit the radio wave (Figure 4). Researchers know the global position of RET by the ARGOS system, approach its area by vessel, then find RET by searching the source of radio wave using the direction finder, and then recover RET. Stored data in RET are retrieved at the laboratory. If the tagged salmon come to the river before RET release, RET is recovered by a radio station settled at riverside.

DISCUSSION

Location of fish is necessary to analyze the migration and swimming speed of fish. Location of marine mammals attached to PTTs can be collected by the ARGOS system when they surface. But radio wave transmitted in the seawater does not reach the satellite, so that fish location is not viewed by the ARGOS system. DeLong et al. (1992) estimate the location of marine animals using day length. Gunn et al. (unpubl. data) examined the behavior of southern bluefin tuna using light intensity in sea water. The location of salmon in the ocean is considered to be able to measure by light intensity. Sensor of light intensity is possible to be installed to the intelligent tag.

Salmons are affected by external acoustic tag on the growth and swimming performance (Lewis and Muntz 1984, Greenstreet and Morgan 1989). Plaice attached the ultrasonic tag would be expected to slow down by 5 to 7%, if the tag weighed 3–4 g in seawater (Walker et

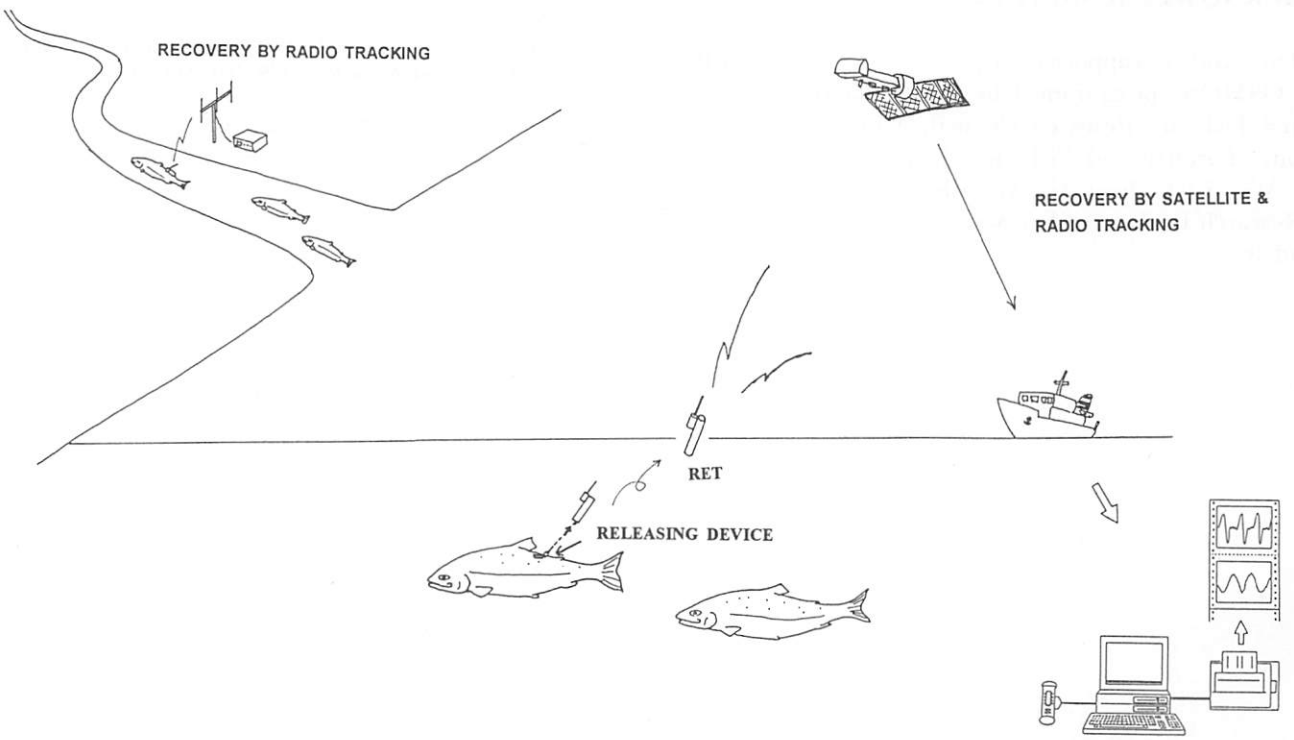

Figure 4. Performance image of RET (recovery-type electric tag).

al. 1978). Although RET has no weight in seawater, the volume of RET seems to impede the fish. RET must be as small as possible.

Location error of PTTs are about 150 m on best receiving condition (Taillade 1992), so that we can approach RET in the ocean. Although we use either Argos direction finder or VHF direction finder to find RET, we find RET by sight at final stage of recovery. We could recover the prototype RET (3.5 cm diameter ×21.8 cm length) off northern Japan (Figure 3). But if RET were smaller, its discovery would get more difficult. Dyeing powder or a balloon inflated with gas etc. may be necessary. Trawling the sea surface may also be useful in recovering RET. Direction of RET changes variously because both vessel and RET are pitching and rolling. Radio wave can be received more accurately when the receiver is set higher. Use of a helicopter set the receiver may better find RET on sea (Kenward 1987).

It is important to avoid harming the animals for study. All of the telemetry instruments must be released, if the research purpose is to be achieved. At this point RET may be one of the most useful techniques for studying the behavior of wild animals and fishes. RET has the potential for re-use. Although the present devices must be smaller, RET is considered to be a useful technique to examine the behavior of fish in the ocean.

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LITERATURE CITED

DeLong, R. L., and B. S. Stewart. 1991. Diving patterns of northern elephant seal bulls. Marine Mammal Science 7: 369–384.

DeLong, R. L., B. S. Stewart and R. D. Hill. 1992. Documenting migrations of northern elephant seals using day length. Marine Mammal Science 8: 155–159.

Fretwell, M. R. 1989. Homing behavior of adult sockeye salmon in response to a hydroelectric diversion of home stream waters at Seton Creek. Int Pacific Salmon Fish Comm, Bull 25, 23–38.

Greenstreet, S. P. R., and R. I. G. Morgan. 1989. The effect of ultrasonic tags on the growth rates of Atlantic salmon, *Salmo salar* L., parr of varying size just prior to smolting. J. Fish Biol. 35, 301–309.

Gunn, J., T. Polacheck, T. Davis, A. Betlehem and M. Sherlock. 1994. Archival tagging of southern bluefin tuna 1993–94 report. Documents submitted to the 6th SBT Recruitment Monitoring Workshop 1994, RMWS/94/No.10. CSIRO, 12 pp. (unpublished report)

Kenward, R. 1987. Wildlife Radio Tagging; Equipment, Field Techniques and Data Analysis. Academic Press. London. 222 pp.

Lewis, A. E., and W. R. A. Muntz. 1984. The effects of external ultrasonic tagging on the swimming performance of rainbow trout, *Salmo gairdneri* Richardson. J. Fish Biol. 25, 577–585.

Naito, Y., T. Asaga and Y. Ohyama. 1990. Diving behavior of Adelie penguins determined by time-depth recorder. The Condor, 92(3). 582–586.

Ogura, M., and Y. Ishida. 1992. Swimming behavior of coho salmon, *Oncorhynchus kisutch*, in the open sea as determined by ultrasonic telemetry. Can. J. Fish. Aquat. Sci. 49, 453–457.

Potter, E. C. E., D. J. Solomon and A. A. Buckley. 1992. Estuarine movements of adult Atlantic salmon (*Salmo salar* L.) in Christchurch Harbour, southern England. In: I. G. Priede and S. M. Swift (eds.), Wildlife Telemetry, Remote Monitoring and Tracking of Animals, p. 400–409. Ellis Horwood Limited, England.

Taillade, M. 1992. Animal tracking by satellite. In: I. G. Priede and S.M.Swift (eds.), Wildlife Telemetry, Remote Monitoring and Tracking of Animals, p. 149–160. Ellis Horwood Limited, England.

Walker, M. G., F. R. H. Jones, and G. P. Arnold. 1978. The movements of plaice (*Pleuronectes platessa* L.) tracked in the open sea. J. Cons. int. Explor. Mer. 38(1), 58–86.

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TIME-RESOLVED FLUOROIMMUNOASSAY (TR-FIA): MEASUREMENT PRINCIPLE AND APPLICATION FOR SALMON RESEARCH

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INTRODUCTION

Precise analysis of experimental data is necessary for elucidation of various biological events. Recent improvement of analytical technique for various biological substances has still contributing to the progress of life science. These methods are used for characterization and evaluation of endocrinological, physiological, ecological, and general biological events by research scientists.

Since the radioimmunoassay (RIA) technique has been introduced and established, it has been used world wide for the measurement of small amounts of substances. The RIA method, which is relatively easy operation and gives precise results, has contributed to endocrinological improvement. This method, however, requires the use of radioisotope, which causes many inconvenience (harmful radiation, such as α-, β-, γ-, X-rays) to human health and the environment. Moreover, the severe regulations of radioisotope do not permit the use of these materials and experiments in a conventional experiment room or area, indicating the necessity of a specially designed room (radioisotope room: RI room). Only a few scientists can operate these facilities, but not others even though they want to use radioisotope. This has been retarding rapid improvement of life science and other science fields.

Enzyme immunoassay (EIA) method has been developing to improve the weak features of the RIA. The EIA method shows a various positive character such as low running cost and safety to the scientists and environment. However, the system still has several weak features, such as background noise, relatively lower sensitivity, and difficulties of long-term storage of enzyme-labeled antigen or antibody.

Fluoroimmunoassay (FIA) uses fluorescence substance (such as FITC) as tracer, instead of enzyme in EIA. Sensitivity of FIA is theoretically 10^2–10^3 times higher than EIA. Time-resolved fluoroimmunoassay (TR-FIA), which is a kind of FIA method, has been developed to improve the weak points of EIA (see above). In this method, fluorescence is measured after disappearance of the background noise (Figure 1). Therefore, this method is not influenced by the noise, resulting enhancement of detection and higher sensitivity. Detectable range of the fluorescence of this system is between 10^{-7}– 10^{-14} mol/L, and the detection sensitivity is almost the same as the ^{125}I method or less.

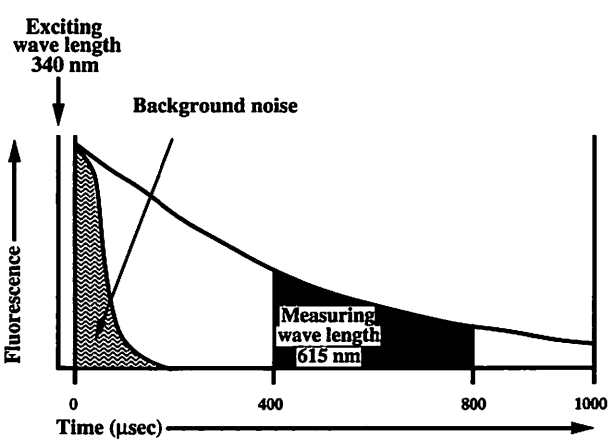

Figure 1. Measurement theory and fluorescence character of Eu in the time-resolved fluoroimmunoassay (TR-FIA).

Nikko branch of the National Research Institute of Aquaculture, is located in Nikko National Park, where it is difficult to use radioactive materials and its disposal. We are developing the TR-FIA system for measurement of hormones to elucidate the function of hormones on migratory behavior of salmonid fish. In this paper, we introduce the principle of measurement and application for salmon research of the TR-FIA. We hope that the

non-RI system (such as TR-FIA) will spread all over the world, and take the place of RIA in the near future for safety and convenient research.

MEASUREMENT PRINCIPLE

Generally, there are two major assay methods (competitive or non-competitive assay) in the immunoassay. Immunoreaction is occurred between specific antibody (Ab), labeled antigens (Ag*) and unlabeled antigens (Ag).

The immunoreaction of competitive assay reaches equilibrium by adding unlabeled antigens. The amount of bound Ag* (Ab-Ag* complex) decrease, and free Ag* (unbound Ag*) increase in the equilibrium phase, resulting in a displacement curve between bound Ag* and free Ag* in this system (Figure 2). Therefore, increasing Ag amount causes decreasing bound Ag*, and decreasing Ag amount results increasing bound Ag*. This method, generally, is employed for the immunoassay of small molecule substances (such as steroid and thyroid hormone), of which antigenic determinant is only one (Figure 3).

Generally, serum hormone concentrations in the salmonid fish are higher at reproductive phase and lower at non-reproductive phase than other animals and humans. Measuring sensitivity of commercially available TR-FIA kit is lower than conventional RIA for serum sample of salmonid fish, suggesting that the kit is not available for the measurement of hormones in the salmonid fish. In our method, we tried to obtain higher sensitivity using avidin-biotin method for the antigen-antibody reaction for testosterone TR-FIA (see labeling method in Figure 4). The biotin-labeled testosterone is synthesized from testosterone-3-CMO, biotin, and 1,3-diaminopropane (DAP) or putrescine as spacer between the testosterone and biotin molecule (Figure 5). Because of the difference of antigenic structure of the labeled testosterone, biotin-labeled

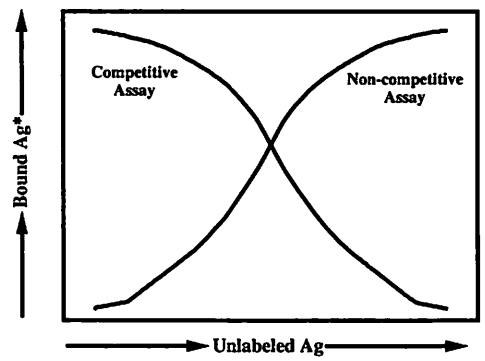

Figure 2. Theoretical pattern of antigen and antibody reaction in the increasing amount of unlabeled antigen in the immunoassay.

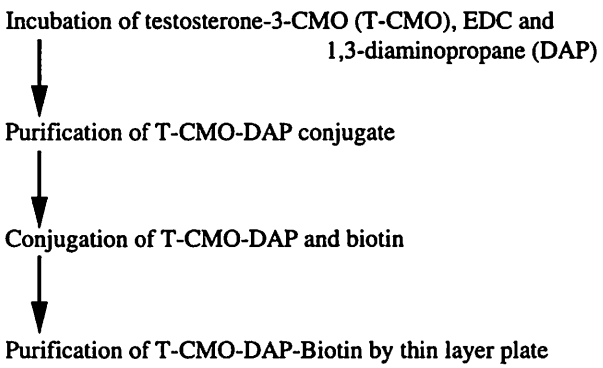

Figure 4. Labeling method for biotin-testosterone conjugate.

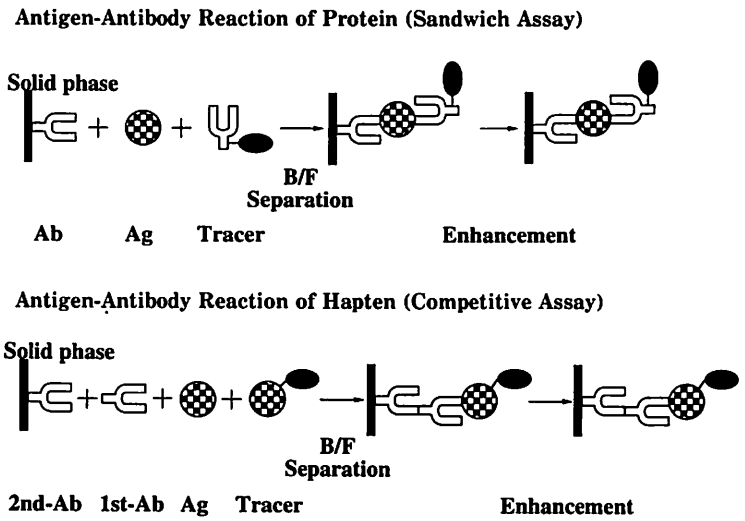

Figure 3. Schematic illustration of two different immunoreactions (sandwich assay and competitive assay).

testosterone without spacer did not react with the testosterone antibody, which was obtained from immunization of bovine serum albumin — testosterone conjugate. Among two types of biotin-labeled testosterone (biotin-DAP-testosterone, biotin-PUT-testosterone), biotin-DAP-testosterone showed higher binding activity for the antibody than biotin-PUT-testosterone (Figure 6).

Figure 7 shows the schematic illustration of competitive assay used in this study. Antibody (anti-testosterone antibody), antigen (unlabeled testosterone), and 1st tracer (biotin labeled testosterone) are incubated with

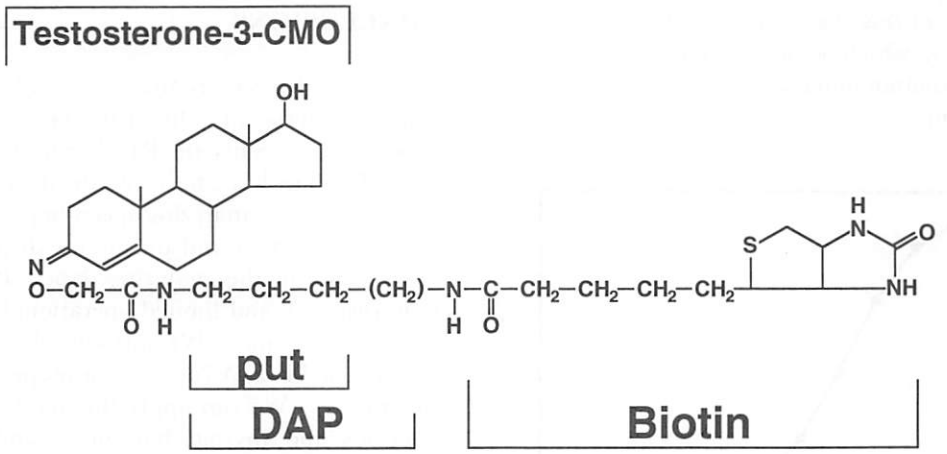

Figure 5. Molecular structure of biotin-labeled testosterone. put: putrescine; DAP: 1,3-diaminopropane.

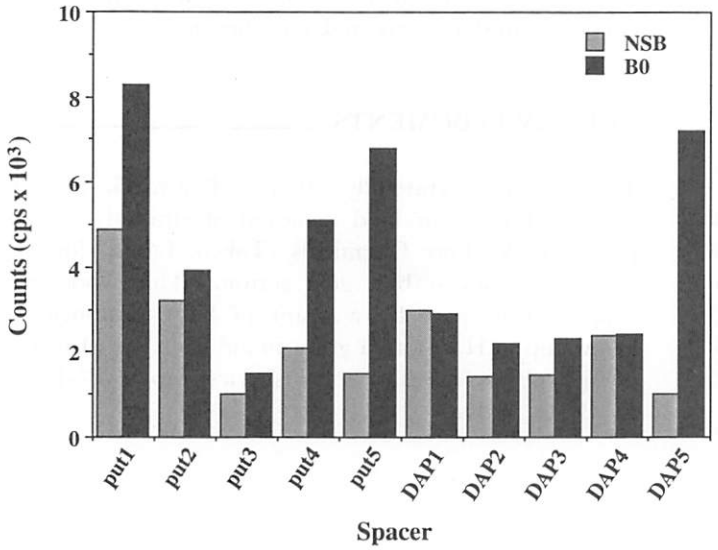

Figure 6. Comparison of non-specific binding (NSB) and total binding (B0) between two spacers (put: putrescine, DAP: 1,3-diaminopropane). Each number after "put" or "DAP" show a band number on TLC plate.

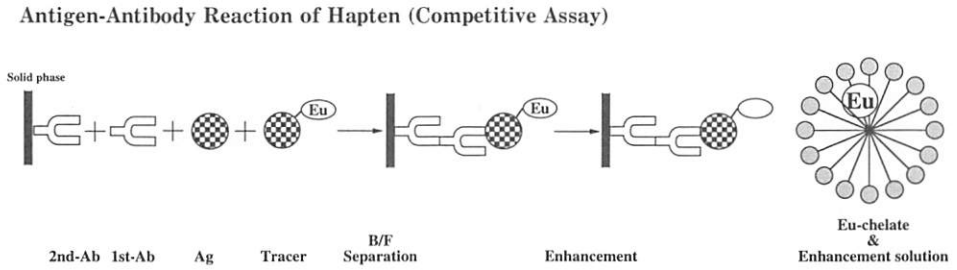

Figure 7. Schematic illustration of testosterone TR-FIA.

the second antibody (anti rabbit IgG goat serum) in a well of microtiter plate. Each well was washed with solution (PBS, 0.01% Tween-20, 0.05% NaN_3) 4 times after 3 h incubation, then Eu-labeled streptavidin was added, incubated for 45 min, and washed. The Eu was extracted with enhancement solution from 2nd antibody, 1st antibody, biotin-testosterone, and Eu-labeled streptavidin complex. Fluorescence activity is counted. Measuring range of this system is from 25 pg/ml to 25 ng/ml (Figure 8), which is wider than the range of conventional radioimmunoassay of testosterone (30 pg/ml to 4 ng/ml).

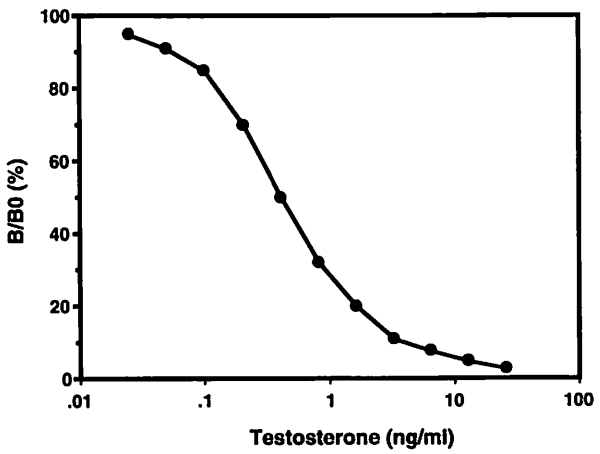

Figure 8. Typical standard curve for testosterone TR-FIA. Increasing amount of unlabeled standard testosterone (25 pg/ml–25 ng/ml) replaced biotin-labeled testosterone.

Recently, a double-labeled method for TR-FIA has been introduced from Wallac (Pharmacia). Because of the narrow energy window between ^3H and ^{14}C, only the RIA has been used for the simultaneous measuring of two different substances. In contrast, two lanthanide elements (Eu and Sm) are used as tracers in the double-labeled TR-FIA (see Table 1).

Table 1. Difference of fluorescence character (wave length and life) between europium and samarium

Fluorescence	Europium	Samarium
Wave length	613 nm	643 nm
Life	730 μsec	50 μsec

CONCLUSIONS

The most ideal environment for biological scientists is rapid analysis of obtained data during or after experiments. Since the RIA has been developed, many scientific data have been obtained in various research fields, and their analyzing speed improved. Except for harmful radiation and dangerous disposal, the RIA is very useful for the analyzing data. Problem of radiation, disposal, and limited operational facility still exist in this technique. We introduced a lot of profitable features of TR-FIA (non-radioisotope immunoassay) in this paper. We can apply this method for both small (steroids and thyroid hormones) and large molecule (proteins) substances with wide dynamic range and sensitivity and without a very low background noise. We hope that the TR-FIA system will spread all over the world in the near future for safety and better environmental reasons and for operators.

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DIURNAL CHANGES IN SCHOOLING BEHAVIOR IN SALMONIDS AND THE ENVIRONMENTAL FACTORS

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ABSTRACT

Many salmonids show schooling behavior throughout their migrating stage regardless of whether they reside in marine or freshwater. Changes in both the incidence of group catches of salmonids and size of the fish group observed in gillnet operations in the Bering Sea demonstrate that salmonids exhibit a diurnal variation in their schooling behavior. An index of schooling behavior, the nearest neighbor distance (NND) between individuals of coho salmon, was analyzed under a non-feeding condition in a light-proof room. Three groups of NND were observed according to the intensities of illumination; the largest NND (1.15 times fork length (FL)) at 0 lx, the medium NND (0.78 to 0.84 FL) at 0.01–0.4 lx, and the least NND (0.60 to 0.66 FL) at 4–4000 lx, but no common periodical rhythm in NNDs was seen in a series of illumination intensities. The NNDs of blinded fish were equal to those of intact fish exposed to 0 lx, while they were significantly larger than those of simulated NNDs calculated from random distribution. These facts suggest that environmental light and vision are important for recognizing other individuals in a school, and that not only vision but also other senses are related to schooling behavior.

INTRODUCTION

Most ecological and physiological studies on salmonids have been based on an individual fish, except for some notable works (Hoar 1954, 1956, 1958; Kobayashi 1958; Ali 1959; Brett and Groot 1963; etc.). As with other migrating fishes, it is well known that salmonids do not live solitarily but aggregate for most of their life (Hoar 1954, 1958). Investigation of schooling behavior, therefore, is important in understanding their unstressed intrinsic biological characteristics. Growth, survival rates, and other important characteristics of fish are known to be controlled by the quality of the fish school (Parker 1973; Clayton 1978; Beacham 1989; Ruzzante and Doyle 1990). In this paper, we summarize the basic schooling behavior of salmonids, which we have been recently carrying out, with special reference to diurnal variation and environmental factors based on experimental studies. In particular, the effect of light on schooling behavior is examined.

DO SALMON EXHIBIT DIURNAL VARIATIONS OF SCHOOLING BEHAVIORS?

In the North Pacific Ocean and the Bering Sea, two or more salmon are often entangled together in a narrow space of a gillnet, an inter-fish distance of ca. 6 meters at the most (Figure 1). Such a "group catch" is commonly observed in gillnet operations. If individual fish are supposed to swim randomly in space, group catch rates are calculated as a function of the fish density. However, the proportion of actual group catches was higher than the expected value which was calculated from a random distribution of fish (Figure 2). This means that salmonids were not entangled individually

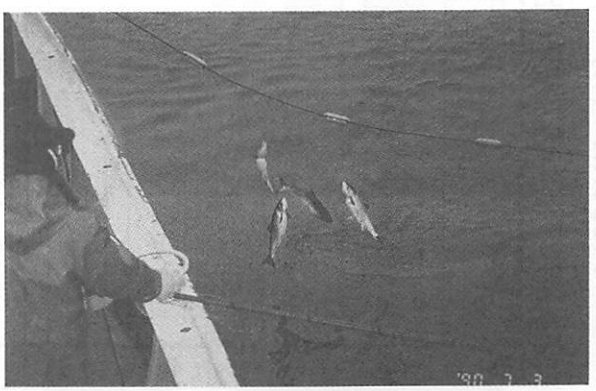

Figure 1. An example of a group catch observed in a gillnet in the North West Pacific Ocean.

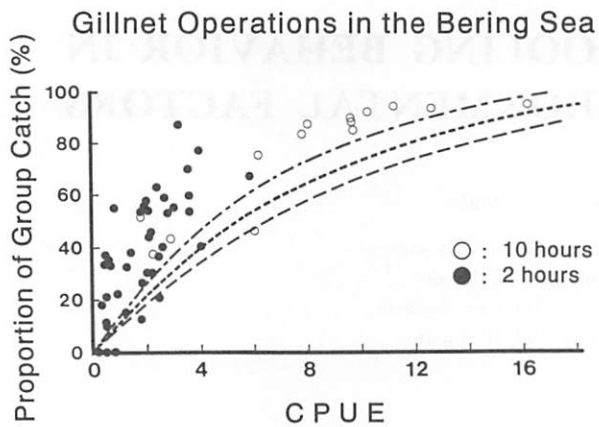

Figure 2. Relationship of the proportion of group catch and fish density shown as the number of fish per tan of gillnet (CPUE). Proportion of group catch equals the proportion of fish caught in a group (comprised of two or more fish in which inter-fish distance is within 6 m) to the total catch. Open and closed circles indicate group catches (%) in gillnet operations whose soaking time are ten and two hours, respectively. A simulated stochastic curve is shown with the 95% confidence interval (dashed lines).

and randomly, but swimming together with others as a group. The group catch as shown in Figure 1 is judged reflecting a school of salmonids. Differences between observed and expected group catches demonstrate the diurnal change of schooling behavior of salmonids (Figure 3). Group catch (%) was higher from evening to midnight, although not always identical between gillnet operations. Also in group size, similar diurnal variations were shown (Figure 4). That is, groups of three or more fish appeared in high proportion from evening to night, although paired groups occupied the highest proportion at any time of the day.

Even in a pond, a sockeye school exhibited a diurnal change in activities (Figure 5). Activities were recorded as counts per hour using a touch sensor which was set in the pond. Changes in activity level corresponded well with that of illumination intensity.

As shown by the examples mentioned above, the diurnal variation in the schooling behavior of salmonids is considered to be associated with environmental

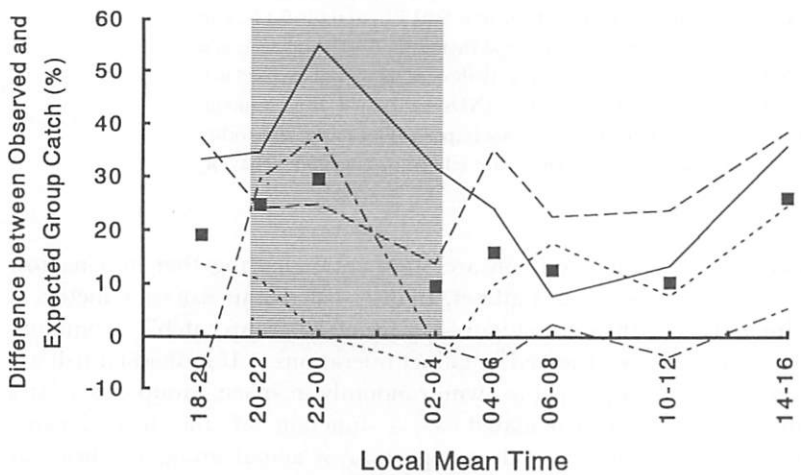

Figure 3. Difference between observed and expected group catch shown as a function of time of day. Expected group catches were calculated using the stochastic curve shown in Figure 2. Hatched area indicates nighttime. The lines show the results of a series of four gillnet operations and the solid squares indicate the overall mean.

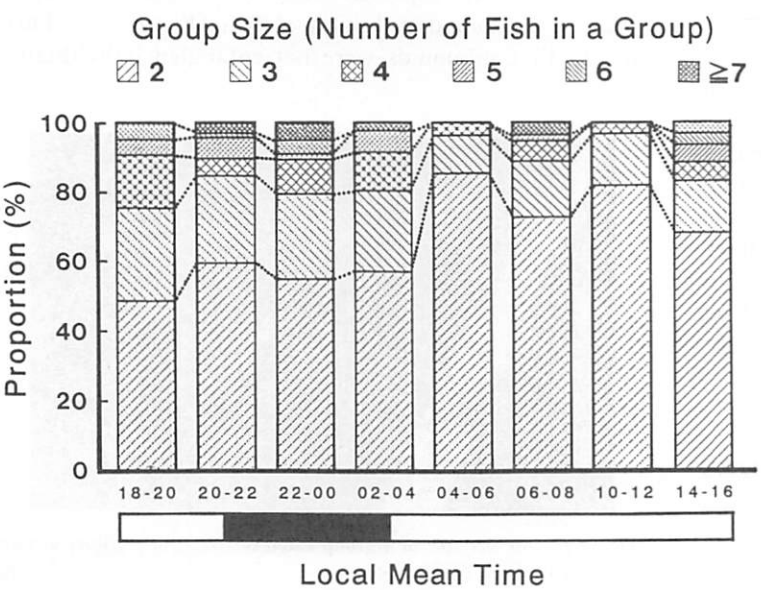

Figure 4. Number of fish in the group shown as a function of time of day. Solid part of the horizontal bar indicates nighttime.

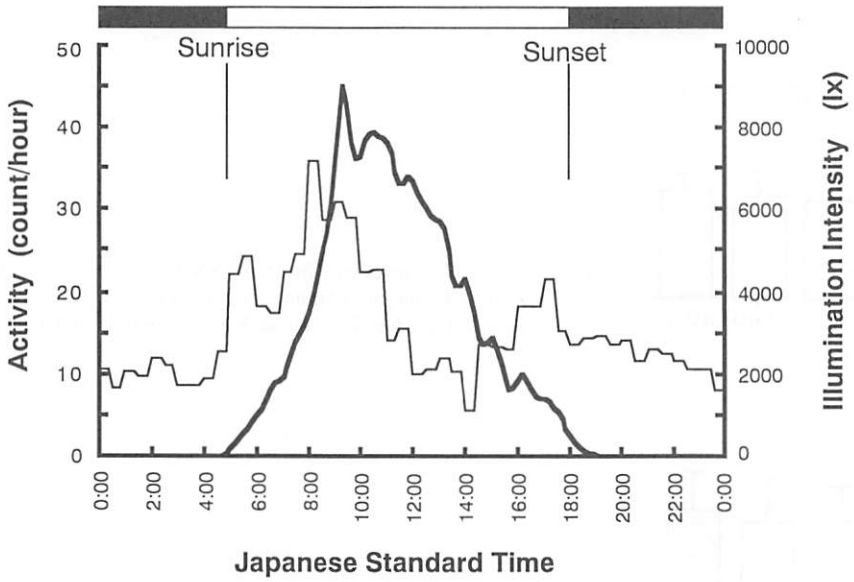

Figure 5. Diurnal change in activity level (fine line) of sockeye schools in a pond compared with illumination intensity (bold line). The horizontal bar shown in upper part of the figure indicates daytime (open) and nighttime (closed).

factors. Of the factors, the illumination intensity, which shows periodical variations in the natural environment, is sure to be an important factor.

EXPERIMENTAL ANALYSIS FOR SCHOOL FORMATION

To understand the behavioral relationship between individuals in a school, the distance to the nearest neighbor (NND) was selected as an index. Coho smolts, which easily show stable schooling behaviors, were used in the experiments. A monitoring system comprising of a video camera and a time recorder was placed above the experimental tank. Experiments were carried out in an indoor tank for examining the effects of light intensities on schooling behavior (Azuma and Iwata 1994). In the experiments, head positions of fish in the tank were converted to two dimensional coordinates from the video records using a video measuring gauge (IV–560, Hoei Co. Ltd), and the NNDs were calculated (Azuma and Iwata 1994).

Under a constant illumination intensity there was no periodical change in NNDs and the fluctuations observed were not associated with the light-dark period before the experiment (Figures 6 and 7). This suggests that the diurnal change in schooling behavior shown under natural photoperiod (Figure 5) is influenced by changes in environmental light intensity. Comparison of NNDs in coho schools between various illumination intensities proved that NNDs were divided into three groups according to the illumination intensities; the largest NND (1.15 times fork length (FL)) at 0 lx, the medium NND (0.78 to 0.84 FL) at 0.01 to 0.4 lx, and the least NND (0.60 to 0.66 FL) at 4 to 4000 lx (Figure 8).

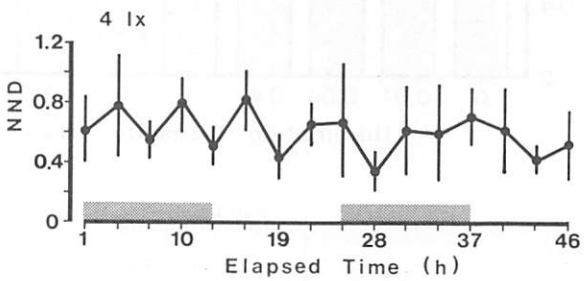

Figure 6. Nearest neighbor distance (NND) observed in coho schools under a constant light intensity of 4 lx. Means and standard deviations are shown. The shaded areas show the light-dark period before the experiment (drawn from Azuma and Iwata 1994).

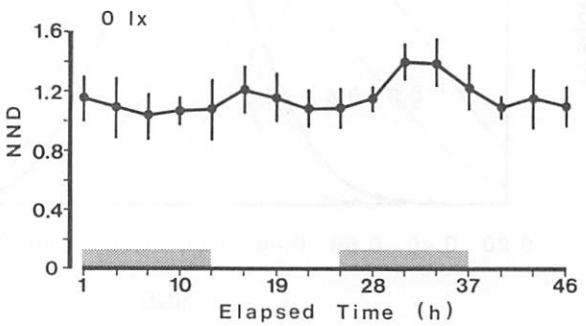

Figure 7. Nearest neighbor distance (NND) observed in coho schools under constant dark condition. Means and standard deviations are shown. The shaded areas show the light-dark period before the experiment (drawn from Azuma and Iwata 1994).

Importance of environmental light was also examined by a vision blocking treatment. Blinded fish exhibited large NNDs irrespective of environmental illumination intensities, almost the same as those of intact fish exposed at 0 lx intensity (Figure 9). Both blinded and

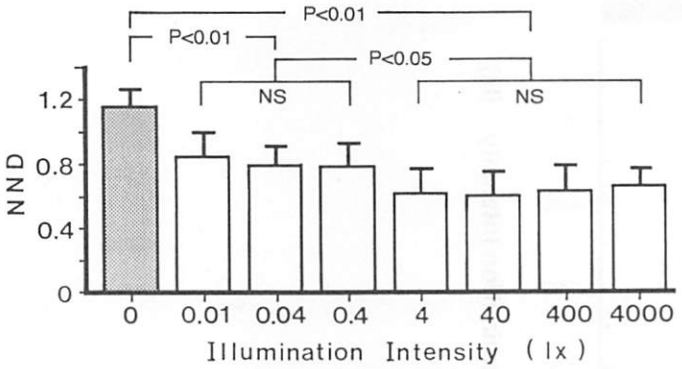

Figure 8. Nearest neighbor distance (NND) observed in coho schools under various illumination intensities. Vertical bars show standard deviations (modified from Azuma and Iwata 1994).

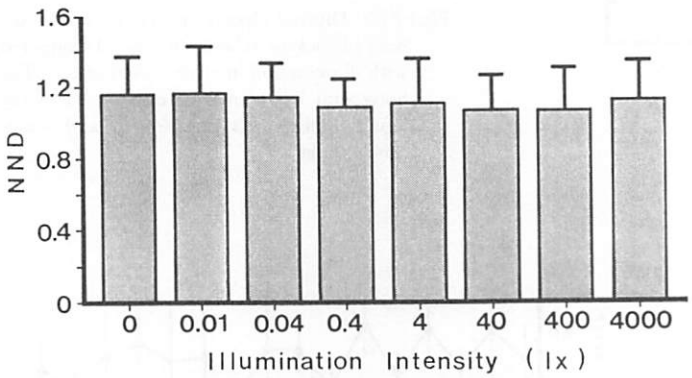

Figure 9. Nearest neighbor distance (NND) observed in blinded coho salmon under various illumination intensities. Vertical bars show standard deviations (from Azuma and Iwata 1994).

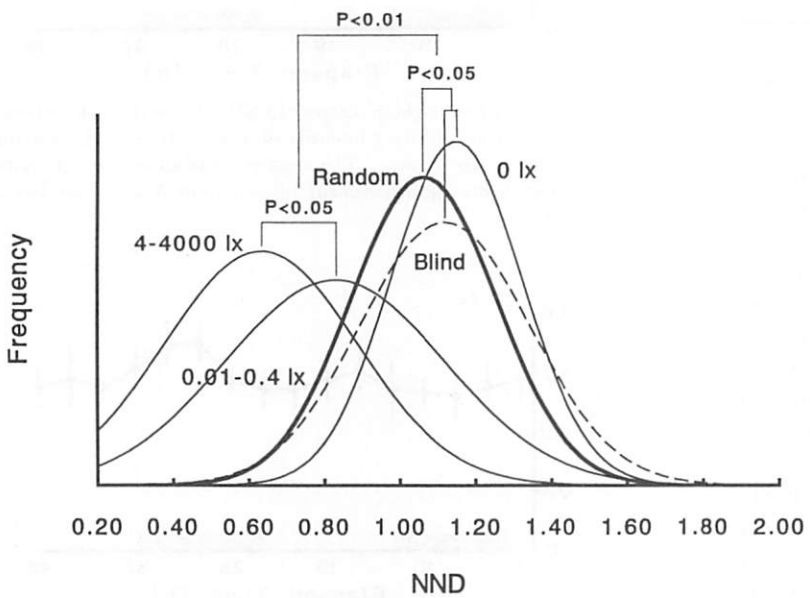

Figure 10. Normal distribution curves of nearest neighbor distance (NND) in coho schools under several light conditions (fine lines) and in blinded coho salmon (dotted line) compared with the curve obtained from a random distribution of fish in an experimental tank (bold line) (modified from Azuma and Iwata 1994).

intact fish exposed at 0 lx showed significantly larger NNDs than simulated NNDs based on random distribution of fish (Figure 10). This means that fish whose visual sense was blocked tended to locate apart from others properly, and thus not only vision but also other senses seem to be associated with recognizing others.

Results obtained from the observations and experiments mentioned above demonstrated that the environmental light condition has important effects on the diurnal schooling behavior of salmonids.

ACKNOWLEDGMENTS

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LITERATURE CITED

Ali, M. A. 1959. The ocular structure, retinomotor and photobehavioral responses of juvenile Pacific salmon. Can. J. Zool. 37: 965–996.

Azuma, T., and M. Iwata. 1994. Influences of illumination intensity on the nearest neighbour distance in coho salmon *Oncorhynchus kisutch*. J. Fish Biol. 45: 1113–1118.

Beacham, T. D. 1989. Effect of siblings on growth of juvenile coho salmon (*Oncorhynchus kisutch*). Can. J. Zool. 67: 601–605.

Brett, J. R., and C. Groot. 1963. Some aspects of olfactory and visual responses in Pacific salmon. J. Fish. Res. Bd. Canada 20: 287–303.

Clayton, D. A. 1978. Socially facilitated behavior. Quart. Rev. Biol. 53: 373–392.

Hoar, W. S. 1954. The behaviour of juvenile Pacific salmon, with particular reference to the sockeye (*Oncorhynchus nerka*). J. Fish. Res. Bd. Canada 11: 69–97.

Hoar, W. S. 1956. The behaviour of migrating pink and chum salmon fry. J. Fish. Res. Bd. Canada 13: 309–325.

Hoar, W. S. 1958. The evolution of migratory behaviour among juvenile salmon of the genus *Oncorhynchus*. J. Fish. Res. Bd. Canada 15: 391–428.

Kobayashi, T. 1958. An ecological study on the salmon fry, *Oncorhynchus keta*. 5. The behaviour of chum salmon fry on their seaward migration. Sci. Rep. Hokkaido Salmon Hatchery 12: 21-30.

Parker, Jr. F. R. 1973. Reduced metabolic rates in fishes as a result of induced schooling. Trans. Amer. Fish. Soc. 102: 125-131.

Ruzzante, D. E., and R. W. Doyle. 1990. Behavioural and growth responses to the intensity of intraspecific social interaction among medaka, *Oryzias latipes* (Temminck and Schlegel) (Pisces, Cyprinodontidae). J. Fish Biol. 37: 663-673.

Bull. Natl. Res. Inst. Aquacult., Suppl. 2: 43-48 (1996)

NEW APPROACHES FOR THE MANIPULATION OF OVULATION AND SPAWNING IN FARMED FISH

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ABSTRACT

Modern and intensive fish farming requires a predictable and reliable supply of fertilized eggs. However, some fish species ovulate and spawn in a non-synchronized and unpredictable manner, while others simply do not ovulate or spawn when raised in captivity. The absence of ovulation and spawning in farmed fish is the result of a failure of the pituitary to release the maturational gonadotropin (GtH-II). Injection of gonadotropin-releasing hormone (GnRH) induces a short surge of GtH-II secretion, but not ovulation. The short-lived effect of the native GnRH is the result of its rapid cleavage by site-specific peptidases located in the pituitary, kidney and liver of the treated fish. Long-acting and super-active analogs of GnRH were identified, based on their increased resistance to degradation, and also their increased affinity to the pituitary receptors. However, these analogs are still cleared from the circulation relatively fast, with half-life times of 15–25 minutes. Therefore, multiple injections of these analogs are necessary to induce ovulation and spawning. Such repetitive injections are labor intensive and stressful to the fish. In order to prolong the presence of the GnRH analogs in the blood of the fish, they were incorporated into polymer-based, controlled-release delivery systems (implants or microspheres). Once injected into the fish, these delivery systems release the GnRH analogs in a sustained manner for periods ranging from 10 days to 2 months, depending on the polymer used and the composition of the devices. These GnRH analog delivery systems are highly efficient in inducing and synchronizing ovulation and spawning in a wide range of farmed fish, with no effect on the quality of the eggs. They can also advance ovulation by 6–8 weeks. In addition, the GnRH analog delivery systems dramatically enhance sperm production.

INTRODUCTION

The intensification and success of aquaculture depend on a regular availability of fertilized eggs and fry. Commercial finfish hatcheries should be able to provide the growers with the required numbers of fertilized eggs at optimal times during the year. Modern and intensive aquaculture cannot depend on the seasonal and unreliable use of gravid broodstock captured on their spawning ground, or on fry collected in the wild. Domesticated broodstocks should be developed for the species of interest, and should provide good quality, genetically superior eggs year-round. However, many farmed fish, including salmonid species, ovulate in a non-synchronized and unpredictable manner, while other species simply do not ovulate or spawn when raised in captivity. Intensive research has been carried out in recent years in an effort to better control ovulation and spawning in farmed fish (for review see Peter et al. 1988; Zohar 1989). Despite these efforts, methods of spawning induction have been only partially successful and did not satisfy the industry requirement. This paper briefly reviews the environmental and hormonal regulation of gametogenesis in fish, focusing on salmonids, and describes the research that led to a new and effective technology to synchronize and induce final oocyte maturation (FOM), ovulation, and spawning in farmed fish.

ENVIRONMENTAL AND HORMONAL CONTROL OF GONADAL DEVELOPMENT

Most fish of temperate zones, such as salmonids, display a seasonal reproductive cycle, with a limited spawning season. The seasonality of the reproductive cycle is determined by the environmental and climatic conditions to which fish are exposed. Photoperiod and

temperature are the main external factors in the control of reproductive seasonality, with one or the other being more important, according to the species. Additional environmental factors, such as lunar cycles, water flow, salinity, and food availability may also contribute to the control of timing of the yearly breeding cycles in some fish. The environmental cues are transduced into endocrine changes that control gametogenesis through mostly unknown mechanisms. The best known transduction mechanism is for photoperiod, which is perceived in fish both through the eyes and photoreceptors of the pineal gland, an endocrine organ which is located on the top of the brain. In response to light-dark changes, the pineal gland synthesizes and secretes the hormone melatonin, which, in turn, participates in determining the timing of gonadal development.

The major endocrine gland that controls gonadal development in fish is the pituitary, or hypophysis. The pituitary gland produces two gonadotropins (GtHs) that act directly at the level of the gonads: GtH-I and GtH-II (Kawauchi et al. 1986; Swanson 1991). The synthesis and secretion of the gonadotropins is regulated by brain neuropeptides named gonadotropin-releasing hormones (GnRHs). Different fish species possess different forms of GnRH, and the number of GnRHs per species ranges from two to three. The brain of most studied fish contains the universal chicken (c) GnRH-II, and a fish-specific form of GnRH, which in many species is salmon (s) GnRH (Sherwood et al. 1994; King and Millar 1995). In some fish, the specific GnRH form is different, such as catfish (cf) GnRH in catfish and mammalian (m) GnRH in eels. Recently, some perciform fish, including gilthead seabream, striped bass, and African cichlid, were shown to possess three forms of GnRH, including a novel fish-specific form, named seabream (sb) GnRH (Powell et al. 1994, 1995; Gothilf et al. 1995). The two other GnRH forms in these fish are cGnRH-II and sGnRH. Despite the multiplicity of GnRHs in fish, it is believed that only one form of GnRH regulates GtH release. This one form may vary from species to species, and is believed to be the fish-specific form, namely sGnRH in salmonid, cyprinids and others, cfGnRH in catfish, mGnRH in eels, and sbGnRH in some perciform fish. The precise role of the other GnRH forms present in fish brains is not yet established. In several species of fish, including cyprinids and catfish, GtH release is subject to dual regulation, a stimulatory effect via the GnRH, and an inhibitory effect via dopamine (Peter et al. 1986).

The relevant GnRH induces the release of both GtH-I and GtH-II. Both gonadotropins induce steroidogenesis in specific gonadal cells. Steroids in turn act at the level of the germ cells and gametes to promote their development. GtH-I is believed to be mostly involved in regulating earlier stages of gametogenesis, i.e. vitellogenesis in females and spermatogenesis in males. Once these processes end, levels of GtH-I in the blood of the fish drop while GtH-II levels rapidly increase. GtH-II is thus believed to be mostly involved in regulating final oocyte maturation and ovulation in the females and spermiogenesis and spermiation in the males (Swanson 1991).

As mentioned above, the GtHs act on the gonads via the mediation of steroids (see for review Fostier et al. 1983; Redding and Patino 1993; Nagahama 1994; Nagahama et al. 1994). In the female, the major reproductive steroids are estrogens (mainly estradiol 17β), which induce vitellogenin production in the liver, and progestagens (mainly 17α, 20β dihydroxy-4-pregnen-3-one and 17α, 20β, 21 trihydroxy-4-pregnen-3-one), which induce final oocyte maturation. In the males, testosterone is the main regulator of spermatogenesis, while 11-ketotestosterone and some progestagens are involved in spermiogenesis and spermiation.

REPRODUCTION IN CAPTIVITY

While in some fish species the reproductive cycle does not seem to be affected by raising the fish in confinement, a number of farmed fish exhibit a wide range of reproductive dysfunctions when held in captivity (Zohar 1989). In the males, while spermatogenesis and spermiation are usually completed, sperm production in captive fish is often reduced as compared to wild fish (e.g. striped bass, some strains of salmon species). In the female of some commercially important farmed fish, such as gilthead seabream, sea bass, striped bass, mullet, grouper and others, oogenesis is not completed in captivity. While the process of vitellogenesis (yolk accumulation) is concluded, final oocyte maturation and ovulation do not occur and thus there is no spawning. Instead, vitellogenic oocytes undergo rapid atresia. In salmonids, final oocyte maturation and ovulation proceed normally in captivity, but the ovulated eggs are not spawned and must be stripped out of the female's body cavity.

It is clear that in order to enable a reliable supply of fertilized eggs from domesticated broodstocks, aquaculture has to overcome the reproduction-related problems encountered in captivity, namely induce and synchronize ovulation and spawning, and enhance sperm production. There are many levels along the environmental-brain-pituitary-gonadal axis where intervention can occur in order to achieve these goals. The most logical level is the environmental one, i.e. simulate the environmental conditions of a spawning ground. However, while this approach has been successful in some cases (e.g. carp species), for many

others it has failed. Many fish species migrate long distances to reach very specific spawning grounds, where they meet a precise combination of external factors that enables spawning. Discovering these combinations is one difficulty, reproducing them in intensive aquaculture conditions is another. A simpler approach is thus to identify the hormonal failure that results from the lack of the appropriate environmental conditions, and devise technologies to overcome the failure and thus induce final gonadal development and spawning. It has been suggested that the absence of FOM and ovulation in some captive fish is the result of a failure to release GtH-II from the pituitary (Zohar 1989). GtH-II accumulates in the pituitary of female seabream held in captivity, but is not released to the circulation, while vitellogenic eggs undergo atresia. These findings led us to focus our research aimed at developing spawning induction technologies on the hormone that normally acts to release GtH-II from the female's pituitary: the GnRH.

GnRH-BASED TECHNOLOGY TO SYNCHRONIZE AND INDUCE OVULATION AND SPAWNING

A single injection of native GnRHs to female seabream undergoing the final stages of vitellogenesis induces an immediate surge of GtH-II release from the pituitary into the blood. However, the duration of the surge is very short (around 6 hr) and no FOM is induced (Zohar 1988). The short-lived effect of the native GnRHs is the result of their very rapid cleavage and inactivation by enzymes present in the pituitary, kidney, and liver of the treated fish (Zohar et al. 1990a). These enzymes specifically break down the native decapeptides (mGnRH and sGnRH) in positions 5–6 and 9–10. Therefore, the first step toward the development of GnRH-based spawning induction therapy was to design GnRH analogs (GnRHa) that are resistant to enzymatic degradation. Substitution of the amino acid glycine at position 6 of the decapeptide with D amino acids, and of the carboxy terminus Gly-NH_2 at position 10 with an N-ethylamide group results in GnRHa that are not recognized by the cleaving enzymes (Zohar et al. 1989; Goren et al. 1990) and are thus longer-acting and super-potent in inducing GtH-II release. In a number of fish, some of these GnRHa have also a higher binding affinity to the pituitary GnRH receptors (De Leeuw et al. 1988; Habibi et al. 1989; Pagelson and Zohar 1992). However, when injected into fish, these highly resistant and super-potent GnRHa still induce a relatively short surge of GtH-II secretion, on the order of 36 hr in seabream (Zohar 1988). The duration of this GtH-II surge is too short to induce FOM, ovulation, and spawning in most studied fish, including salmonid species. Therefore, repetitive GnRHa injections are required to induce successful spawning, which is labor intensive and stressful to the fish. It was shown that the short-term effect of the highly resistant GnRHa is the result of their rapid metabolic clearance from the fish circulation (Gothilf and Zohar 1991). Although the degradation-resistant GnRHa are cleared slower from the fish circulation, as compared to the native peptides, they still have relatively short half-life times (22 min for the analogs as compared to 6 min for the native peptides). This relatively rapid disappearance rate of the GnRHa may suggest that they are cleared from the circulation as intact, uncleaved molecules.

The above research made it clear that in order to efficiently induce FOM, ovulation, and spawning in farmed fish, there is a need to prolong the presence of the GnRHa in the fish circulation, while limiting the hormonal treatment to a single application. To achieve this goal, the highly-resistant and super-potent GnRHa were incorporated into controlled-release delivery systems (Crim et al. 1988; Zohar 1989; Zohar et al. 1990b, 1994; Hodson and Sullivan 1993; Mylonas et al. 1995a). Through a variety of mechanisms, ranging from simple diffusion to biodegradation, these delivery systems release the GnRHa into the blood of the treated fish in a sustained manner. Most of these sustained-release delivery systems are made of polymers, and by varying the chemical composition of the polymers, or by varying the loading percent of the GnRHa or of its inert carrier molecules, the duration and rate of GnRHa release can be modulated. Therefore, these delivery systems can be optimized to fit the gonadal physiology of the fish of interest. The prolonged release of the GnRHa, achieved after a single hormonal application, induces a long-term secretion of GtH-II from the pituitary, which in all studied species results in a highly effective induction of FOM, ovulation, spermiation, and spawning. The GnRHa delivery systems can be prepared either in the form of small implants, that are inserted intramuscularly, or microspheres, that are suspended in a vehicle solution and injected intramuscularly. Figures 1 and 2 show GnRHa release from two different delivery systems, developed for fish with two different spawning strategies, and the GtH-II secretion induced by these two systems. The delivery system presented in Figure 1 was specifically designed to synchronize ovulation in fish that ovulate and spawn only once during the breeding season, such as salmonids. This delivery system results in elevated levels of GnRHa in the blood of treated females for periods of 2–3 wk. The elevated GnRHa levels induce GtH-II release into the fish blood for the same duration (Figure 1). This delivery system was used to induce spawning in a variety of salmonid species, including

rainbow trout, Atlantic salmon, coho salmon, chinook salmon, and sockeye salmon. The results collected from thousands of fish indicate that, when treated 3–4 wk before spawning normally starts, 90–100% of the females treated with the GnRHa delivery system typically ovulate within 12–15 days post-treatment. In the non-treated females, only around 10% ovulate within the same time period, the others ovulating in a non-synchronous manner over 30–60 days (Zohar et al. 1990b,1994; Goren et al., 1995). Moreover, this GnRHa delivery system can be successfuly used to induce ovulation in salmonids 4–6 wk before their usual spawning times. In all cases, egg size, fertilization, eyeing, and hatching rates were not affected by the hormonal treatment.

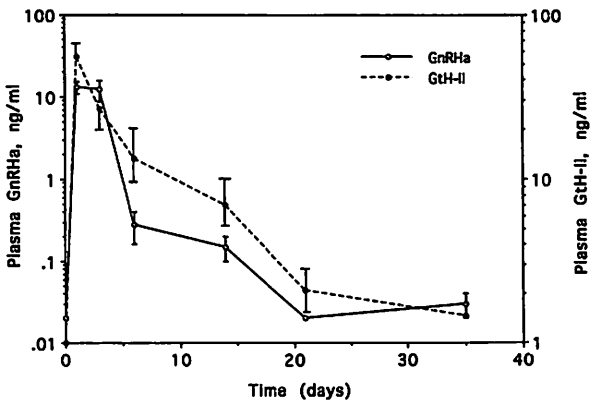

Figure 1. Blood GnRHa and GtH-II levels in female gilthead seabream (n = 8) before and at different times after their treatment with "fast" GnRHa delivery systems.

The delivery system presented in Figure 2 was specifically developed to induce FOM, ovulation, and spawning in fish that spawn multiple times during one breeding season, such as seabream, seabass, grouper species, shad species, and others. In order to obtain repetitive spawning, a more prolonged availability of GnRHa in the blood of the fish was required. Upon administration to the fish, there is an initial surge of GnRHa release to the circulation, followed by slow but continuous release of GnRHa for periods ranging from 35 days (Figure 2) to 2 months (Mylonas et al. 1995a). These elevated GnRHa levels induce GtH-II secretion for the same duration (Figure 2). This delivery system was demonstrated to be very efficient in inducing repetitive spawning in seabream (Zohar et al. 1995), American shad (Mylonas et al. 1995b), and grouper.

Therefore, two different GnRHa controlled-release delivery systems have been developed and are now commercially available: a "faster" release system to be used for single spawners, and a "slower" release system to be used for repetitive spawners. Both GnRHa delivery systems are also highly efficient in enhancing

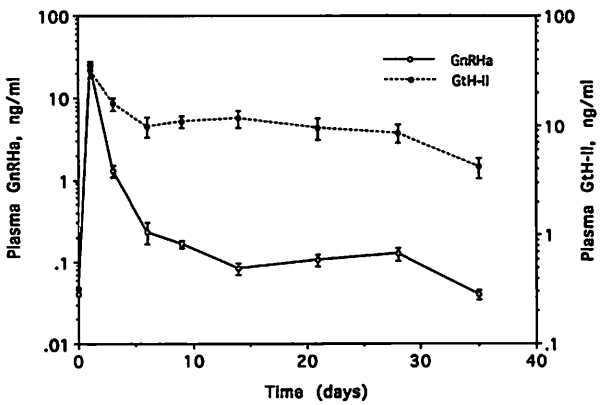

Figure 2. Blood GnRHa and GtH-II levels in female gilthead seabream (n = 8) before and at different times after their treatment with "slow" GnRHa delivery systems.

sperm production in a number of farmed fish. Figure 3 shows sperm production in male Atlantic salmon (n = 40) before and after their treatment with the "faster" GnRHa delivery system. The experimental males were two sea-winter fish, averaging 17.5 kg in body weight. They were males of a fast growing, late maturing strain of Atlantic salmon, which produce abnormally small volumes of sperm. Ten days after their treatment with the GnRHa delivery systems, sperm production in the treated males increased tenfold (Figure 3). Spermatocrit values in the treated males were not significantly different from the controls, indicating that the GnRHa treatment resulted in a stimulation of sperm production, and not only of seminal plasma. The two types of the above-described GnRHa delivery systems have been demonstrated to increase sperm production in a number of additional species, including various species of salmonids, seabream, seabass, striped bass, white bass, and flounder.

The GnRHa controlled-release delivery systems have proven to be an invaluable tool in finfish broodstock management programs in aquaculture. They are

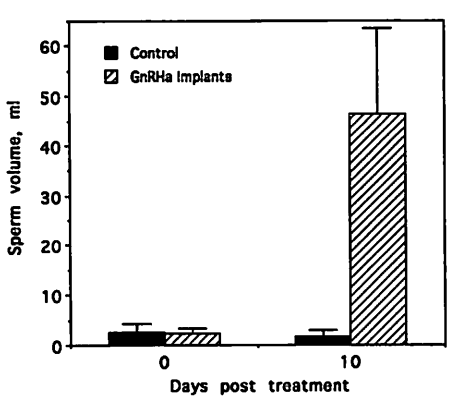

Figure 3: Total volume of sperm stripped from male Atlantic salmon (n = 40) before and 10 days after their treatment with "fast" GnRHa delivery systems.

widely used in commercial finfish hatcheries around the world. They are also presently being experimented within a number of additional food and ornamental fish species. The results reported above demonstrate that the GnRHa delivery systems represent an efficient and generic technology to induce FOM, ovulation, spermiation, and spawning in farmed fish.

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LITERATURE CITED

Crim, L. W., N. M. Sherwood, and C. E. Wilson, 1988. Sustained hormone release. II. Effectiveness of LHRH analog (GnRHa) administration by either single time injection or cholesterol pellet implantation on plasma gonadotropin levels in a bioassay model fish, the juvenile rainbow trout. Aquaculture 74: 87–95.

De Leeuw, R., C. Van't Veer, W. Smit-Van Dijk, H. J. T. Goos, and P. G. W. J. Van Oordt, 1988. Binding affinity and biological activity of gonadotropin-releasing hormone analogs in the African catfish, *Clarias gariepinus*. Aquaculture 71: 119–131.

Fostier, A., B. Jalabert, R. Billard, B. Breton, and Y. Zohar, 1983. The gonadal steroids. (W. S. Hoar, D.J. Randall, and E.M. Donaldson, eds.), In Fish Physiology, Vol IX, "Reproduction" pp. 277–372. Academic Press, New York.

Goren, A., Y. Zohar, M. Fridkin, E. Elhanati, and Y. Koch, 1990. Degradation of gonadotropin releasing hormone in the gilthead seabream, *Sparus aurata*: I. Cleavage of native salmon GnRH, mammalian LHRH and their analogs in the pituitary. Gen. Comp. Endocrinol. 79: 291–305.

Goren, A., H. M. Gustafson, and D. S. Doering, 1995. Field trials demonstrate the efficacy and commercial benefit of a GnRHa implant to control ovulation and spermiation in salmonids. In (R. Goetz and P. Thomas, eds.) Reproductive Physiology of Fish (in press).

Gothilf, Y. and Y. Zohar, 1991. Clearance of different forms of GnRH from the circulation of the gilthead seabream, *Sparus aurata*, in relation to their degradation and bioactivities. In (A. P. Scott, J. P. Sumpter, D. E. Kime, and M. S. Rolfe, eds) Reproductive Physiology of Fish. pp. 35–37. University of East Anglia Press.

Gothilf, Y., A. Elizur, and Y. Zohar, 1995. Three forms of gonadotropin-releasing hormone in gilthead seabream and striped bass: physiological and molecular studies. In (R. Goetz and P. Thomas, eds.) Reproductive Physiology of Fish (in press).

Habibi, H. R., T. A. Marchant, C. S. Nahorniak, H. Van Der Loo, R. E. Peter, J. E. Rivier, and W. V. Vale, 1989. Functional relationship between receptor binding and biological activity for analogs of mammalian and salmon gonadotropin-releasing hormones in the pituitary of goldfish (*Carassius auratus*). Biol. Reprod. 40: 1152–1161.

Hodson, R. G. and C. V. Sullivan, 1993. Induced maturation and spawning of domestic and wild striped bass (*Morone saxatilis*) broodstock with implanted GnRH analog and injected hCG. J. Aquat. Fish. Manage. 24: 271–280.

Kawauchi, H., K. Suzuki, Y. Nagahama, S. Adachi, N. Naito, and Y. Nakai, 1986. Occurrence of two distinct gonadotropins in chum salmon pituitaries. In: (F. Yoshimura and A. Gorbman, eds) Pars Distalis of the Pituitary Gland- Structure, Function, and Regulation. pp. 383–390. Elsevier Press.

King, J. A. and R. P. Millar, 1995. Evolutionary aspects of gonadotropin-releasing hormone and its receptor. Cell. Mol. Neurobiol. 15: 5–23.

Mylonas, C. C., Y. Tabata, R. Langer, and Y. Zohar, 1995a. Preparation and evaluation of polyanhydride microspheres containing gonadotropin-releasing hormone (GnRH), for inducing ovulation and spawning in fish. J. Cont. Rel. 35: 23–34.

Mylonas, C. C., B. M. Richardson, S. P. Minkinnen, and Y. Zohar, 1995b. Induced spawning of American shad (*Alosa sapidissima*) using sustained administration of gonadotropin-releasing hormone analog (GnRHa). J. World Aquacult. Soc. 26: 39–50.

Nagahama, Y., 1994. Endocrine regulation of gametogenesis in fish. Int. J. Dev. Biol. 38: 217–229.

Nagahama, Y., M. Yoshikuni, M. Yamashita, and M. Tanaka, 1994. Regulation of oocyte maturation in fish. In: (A. P. Farrell, and D.J., eds. Randall) Fish Physiology. Vol. XIII: 393–439 (Molecular Endocrinology of Fish, Sherwood, N. M. and Hew C.L., eds). Academic Press, New York.

Pagelson, G. and Y. Zohar, 1992. Characterization of gonadotropin-releasing hormone (GnRH) receptors in the gilthead seabream (*Sparus aurata*). Biol. Reprod. 47: 1004–1008.

Peter, R. E., J. P. Chang, C. S. Nahorniak, R. J. Omeljaniuk, M. Sokolowska, S. H. Shih, and R. Billard, 1986. Interactions of catecholamines and GnRH in regulation of gonadotropin secretion in teleost fish. Rec. Prog. Horm. Res.: 42: 513–548.

Peter, R. E., H. R. Lin, and G. Van Der Kraak, 1988. Induced ovulation and spawning of cultured freshwater fish in China: advances in application of GnRH analogs and dopamine antagonists. Aquaculture 74: 1–10.

Powell, J. F. F., Y. Zohar, M. Park, W. H. Fischer, A. G. Craig, J. F. Rivier, D. A. Lovejoy, and N. M. Sherwood, 1994. Three forms of gonadotropin-releasing hormone characterized from brains of one species. Proc. Natl. Acad. Sci. USA 91: 12081–12085.

Powell, J. F. F., W. H. Fischer, M. Park, A. G. Craig, J. F. Rivier, S. A. White, R. C. F. Francis, R. D. Fernald, P. Licht, C. Warby, and N. M. Sherwood, 1995. Primary structure of a solitary form of gonadotropin-releasing hormone (GnRH) in cichlid pituitary: three forms of GnRH in brain of cichlid and pumpkinseed fish. Reg. Peptides 57: 43–53.

Redding, J. M. and R. Patino, 1993. Reproductive physiology. In: (D. H., edt. Evans) The physiology of fishes. pp: 503–534. CRC Press, Boca Raton, FL.

Sherwood, N. M., D. B. Parker, J. E. McRoy, and D. W. Lescheid, 1994. Molecular evolution of GHRH and GnRH. In: (A.P. Farrell, and D.J. Randall eds.) Fish Physiology. Vol. XIII: 3–66 (Molecular Endocrinology of Fish, Sherwood, N. M. and Hew C. L., eds). Academic Press, New York.

Swanson, P., 1991. Salmon gonadotropins: reconciling old and new ideas. In (A. P. Scott, J. P. Sumpter, D. E. Kime and M. S. Rolfe, eds) Reproductive Physiology of Fish. pp. 2–7. University of East Anglia Press.

Zohar, Y., 1988. Gonadotropin releasing hormone in spawning induction in teleosts: basic and applied considerations. In: (Y. Zohar and B. Breton, eds.) Reproduction in Fish—Basic and Applied Aspects in Endocrinology and Genetics., pp. 47–62.

INRA Press, Paris.

Zohar, Y. 1989. Fish Reproduction: Its Physiology and Artificial Manipulation. In: (M. Shilo and S. Sarig, eds.) Fish Culture in Warm Water Systems: Problems and Trends. pp. 65–119. CRC Press, Boca Raton, Fl.

Zohar, Y., A. Goren, M. Tosky, G. Pagelson, D. Leibovitz, and Y. Koch, 1989. The bioactivity of gonadotropin-releasing hormones and its regulation in the gilthead seabream, *Sparus aurata: in vivo* and *in vitro* studies. Fish. Physiol. Biochem. 7: 59-67.

Zohar, Y., A. Goren, M. Fridkin, E. Elhanati, and Y. Koch, 1990a. Degradation of gonadotropin releasing hormone in the gilthead seabream, *Sparus aurata*: II. Cleavage of native salmon GnRH, mammalian LHRH and their analogs in the pituitary, kidney, and liver. Gen. Comp. Endocrinol. 79: 306–319.

Zohar, Y., G. Pagelson, Y. Gothilf, W.W. Dickhoff, P. Swanson, S. Duguay, W. Gombotz, J. Kost, and R. Langer, 1990b. Controlled release of gonadotropin releasing hormones for the manipulation of spawning in farmed fish. Proceed. Int. Symp. Control. Rel. Bioact. Mater. 17: 51–52.

Zohar, Y., D. Doering, and R. Langer, 1994. Applications of controlled release technology in aquaculture. Cont. Rel. Bioact. Mater. 21: 110–111.

Zohar, Y., M. Harel, S. Hassin, and A. Tandler, 1995. Broodstock management and manipulation of spawning in the gilthead seabream, *Sparus aurata*. In: (N. R. Bromage and R. J. Roberts, eds.) Broodstock Management and Egg and Larval Quality pp. 94–114. Blackwell Press (London, UK).

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STRATEGIES FOR STUDYING THE OLFACTORY MECHANISM IN SALMON HOMING

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ABSTRACT

It is widely believed that the juvenile salmon at the smolt stage imprint to the odor of their home stream. As adults, they use this imprinted olfactory memory to return to their natal stream ("olfactory imprinting hypothesis" by Hasler and Wisby, 1951). Many behavioral experiments support this hypothesis. However, attempts to find neural correlates of the imprinted olfactory memory of the home stream using neurophysiological methods have not yet been succeeded, or have still been a matter of debate. On the other hand, recent progress in the study of learning and memory in higher vertebrates revealed the molecular basis of them. For example, activation of the NMDA receptor is shown to be one of the initial steps responsible for the long-term memory formation. Moreover, neural mechanism of olfactory imprinting was proposed in the accessory olfactory bulb of the mouse. These recent results from the higher vertebrates seem to be very suggestive for the study of neural basis of the salmon homing. Another important approach to the olfactory mechanism of the salmon homing may be to record and analyze neural activities from the fish actually selecting their home stream during spawning migration. For this purpose we have developed an underwater radiotelemetry technique for transmitting the central olfactory nervous activity under freely behaving condition. By using this telemetry technique, we have recently succeeded in recording the olfactory bulbar EEG activity from freely behaving fish in natural environments.

As is well-known, Hasler and Wisby (1951) proposed the "olfactory imprinting hypothesis" for salmon homing. This hypothesis is based on the assumption that (1) each stream has a unique chemical composition, thus, a distinctive odor; (2) before juvenile salmon migrate to the sea they become imprinted to the distinctive odor of their home stream; and (3) adult salmon use this information as a cue for homing towards their natal tributary. Here, "imprinting" is a process of rapid, irreversible learning of a particular olfactory stimulus that occurs at a "critical" or "sensitive" period during development and that influences the behavior of the animal (Hasler et al. 1978; Hasler and Scholz 1983).

The various experimental tests of this hypothesis and attempts to clarify the neural mechanisms of the olfactory imprinting are summarized in this paper. Then, we consider some strategies for studying the olfactory mechanism in salmon homing.

Since the proposal of olfactory imprinting hypothesis by Hasler and Wisby (1951), extensive behavioral experiments, such as (1) sensory-impairment experiments (Wisby and Hasler 1954; Lorz and Northcote 1965; Hiyama et al. 1967; Groves et al. 1968; Bertmer and Toft 1969; Shirahata 1969), (2) transplantation experiments (Donaldson and Allen 1957; Peck 1970; Jensen and Duncan 1971), and (3) artificial imprinting experiments (Scholz et al. 1976; Johnsen and Hasler 1980; Hassler and Kucas 1988), performed by many research groups, have strongly supported this hypothesis (for reviews see Harden Jones 1968; Hasler et al. 1978; Hasler and Scholz 1983; Thorpe 1988).

Among the behavioral experiments, the transplantation experiments by Donaldson and Allen (1957) and by Jensen and Duncan (1971) were important. Jensen and Duncan (1971) transplanted coho salmon smolts from their original hatchery on the Columbia River to a spring-fed fish-holding facility on the Snake River. The fish were held for 2 days and then released into the Snake River. During the spawning migration, marked fish were recovered near the springwater discharge 0.8 km downstream from the release point but not from other locations. To determine if the fish were actually homing to the water in which they had been held as smolts, water from the holding facility was pumped into a floating trap. As a control, river water was pumped into the trap. No fish entered the trap when river water was pumped, but a number of fish were captured when springwater was used. Springwater was thus the

orienting stimulus; fish were able to learn the characteristics of this water within 2 days.

The artificial imprinting experiment performed by Scholz et al. (1976) was also decisive. They exposed the juvenile coho salmon to synthetic chemicals (morpholine or phenyl ethyl alcohol) for 1 1/2 months and released them in Lake Michigan. During the spawning migration 18 months later, morpholine and phenyl ethyl alcohol were metered into separate streams, and the number of morpholine- and phenyl ethyl alcohol-exposed fish returning to each stream was counted. The majority of the fish exposed to morpholine were captured in the stream scented with morpholine and most fish exposed to phenyl ethyl alcohol were captured at the phenyl ethyl alcohol-treated stream. The results show that coho salmon imprint to and utilize artificial chemical cues for homing.

It is generally accepted that the juvenile salmon at the smolt stage imprint to the odor of their home stream. As adults, they use this imprinted olfactory memory to return to their natal tributary, where they spawn and die. This does not necessarily mean that the olfaction is the only sense involved in the salmon homing. There still remains a possibility that other senses, such as vision, play some roles. Thyroid hormones, which control the process of smolt transformation, are believed to facilitate the process of olfactory imprinting (for reviews see Hasler and Scholz 1983; Quinn 1990).

On the other hand, attempts to find neural correlates of the imprinted memory of the home stream odor using neurophysiological methods have not yet been succeeded, or have still been a matter of debate. For example, Hara et al. (1965) and Ueda et al. (1967) reported that water from the home sites evoked a remarkable electroencephalographic response (EEG response) from the olfactory bulb (the primary olfactory center) of the coho salmon, but water from the non-home sites evoked substantially no response.

These observations suggested that the imprinted olfactory memory for the home water was stored in the olfactory bulb, and this stored memory was recalled by the home water stimulation as adults. They proposed that the recalled olfactory memory was represented as the olfactory bulbar EEG response (Hara et al. 1965; Ueda et al. 1967; Hara 1970). However, these results were not confirmed in later experiments by Ueda's own group (Ueda et al. 1971; Kaji et al. 1975) as well as by Bodznick (1975).

About 10 years later, Cooper and Hasler (1974) reported that morpholine, an artificial chemical stimulant, evoked a larger EEG response in the morpholine-imprinted coho salmon than the non-imprinted salmon. However, their results were criticized by Hara's group (Hara and MacDonald 1975; Hara and Brown 1979). Hara and colleagues argued that "the morpholine may penetrate deep into the olfactory epithelium and cause a nonspecific irritational effect at nonspecialized cell surfaces".

Very recently, Nevitt et al. (1994) provided evidence for a peripheral olfactory memory in artificially imprinted salmon. They exposed coho salmon to phenyl ethyl alcohol for 10 days during the parr-smolt transformation, and measured phenyl ethyl alcohol responses in the peripheral receptor cells about 6 months later. They found that cells from imprinted fish showed an increased sensitivity compared to cells from naive fish. Accordingly, they proposed that some components of the imprinted home stream memory are retained peripherally in the olfactory receptor cells. This idea of peripherally stored olfactory memory contrasts to that of the centrally stored memory as noted before, and awaits future study.

Thus, sufficient evidences supporting the olfactory hypothesis of salmon homing have been provided from a number of behavioral experiments, but the neural correlates of the imprinted olfactory memory have not yet been established in spite of studies performed by many researchers over a long period of time. Moreover, neural mechanism of the olfactory memory formation, or olfactory imprinting, still remains unknown. This implies how difficult is the proper understanding of the olfactory mechanism of the salmon homing.

On the other hand, recent progress in the study of learning and memory in higher vertebrates revealed the molecular mechanism of learning and memory (for reviews see Kandel and O'dell 1992; Kandel and Hawkins 1992; Shatz 1992; Bliss and Collingridge 1993). Associative "long-term memory" is believed to be stored in "Hebbian type" synapse. In Hebbian type synapse, plastic changes occur when both the pre- and postsynaptic elements are simultaneously activated. One of the molecules that detects this simultaneous activation is shown to be the "NMDA receptor", a type of glutamate receptors. Glutamate is a representative excitatory neurotransmitter distributed widely in the brain. It is proposed that activation of NMDA receptor is one of the initial steps responsible for the long-term memory formation (Figure 1).

Another suggestive example for olfactory imprinting comes from the study of the accessory olfactory bulb of mice. Female mice form an olfactory memory of male pheromones at mating. Exposure to the pheromones of a strange male after mating will block pregnancy. This is known as the "Bruce effect". The formation of this memory is mediated by the accessory olfactory system, in which an increase in norepinephrine level during the critical period after mating is necessary (Brennan et al. 1990). It is suggested that the olfactory memory formation in the accessory olfactory bulb requires the excitation of both the granule cells and the mitral cells

(through Hebbian type learning) by means of non-NMDA receptor and mGluR2 (other types of glutamate receptor than NMDA type) (Brennan et al. 1990; Hayashi et al. 1993; Kaba et al. 1994) (Figure 2).

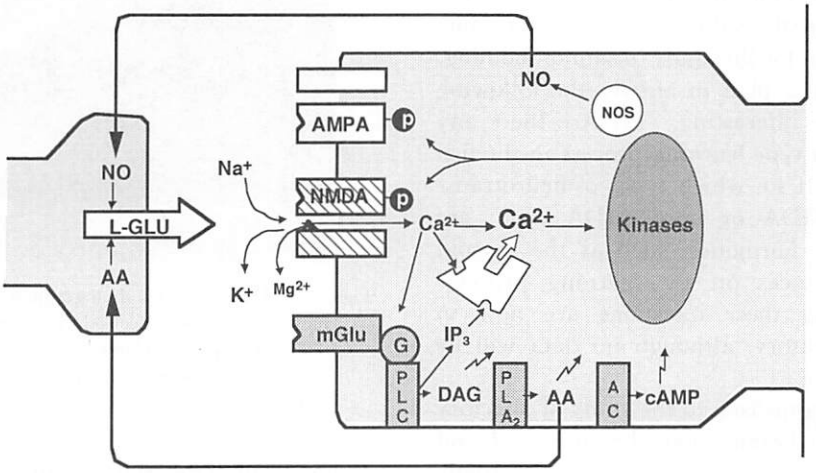

Figure 1. A proposed mechanism for the long-term potentiation in mammalian synapse. In long-term potentiation (LTP) the postsynaptic membrane is depolarized by the actions of the non-NMDA (AMPA) receptor channels. The depolarization relieves the Mg^{2+} blockade of the NMDA-channel, allowing Ca^{2+} to flow through the channel. The initial induction signal for LTP is this Ca^{2+} transient. This signal is then amplified by the release of Ca^{2+} from Ca^{2+}/IP_3-sensitive intracellular stores. A parallel pathway which may be important for the induction of LTP is provided by mGluRs (metabotropic glutamate receptors). These receptors can couple, through G-proteins, to the phosphoinositide-specific phospholipase C (PLC), phospholipase A_2 (PLA_2) and adenylate cyclase (AC), to produce diacylglycerol (DAG), arachidonic acid (AA), and to regulate the levels of cAMP, respectively. Note that the initial NMDA receptor-mediated Ca^{2+} transient may be necessary for the activation of these mGluR cascades by L-glutamate. The amplified Ca^{2+} signal, in association with the other activators of protein kinases (zig-zag arrows), then leads to the phosphorylation of substrate proteins including, probably, AMPA and NMDA receptors. Other enzymes, such as nitric oxide synthase (NOS), if present, may also be activated by the Ca^{2+} transient. Biochemical changes in the presynaptic terminal may be initiated by the action of retrograde messengers, such as arachidonic acid (AA), nitric oxide (NO) and K^+, perhaps in conjunction with the action of L-glutamate on presynaptic mGluRs. (From Bliss and Collingridge, 1993).

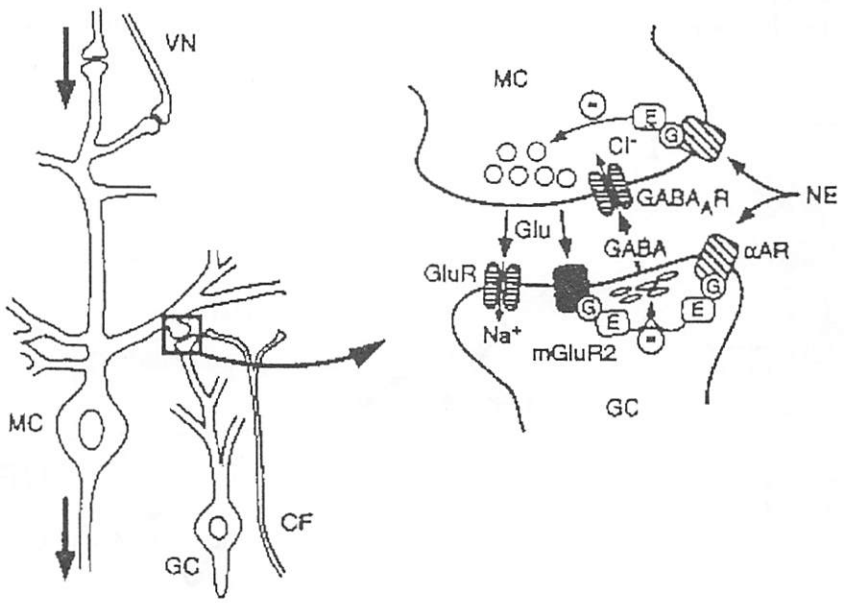

Figure 2. A model for the olfactory imprinting in the accessory olfactory bulb of mice. The olfactory memory of male pheromones is formed in the accessory olfactory bulb, in which an increase in norepinephrine (NE) after mating reduces inhibitory transmission of GABA from the granule cells (GCs) to mitral cells (MCs). Activation of mGluR2, a metabotropic glutamate (Glu) receptor that suppresses the GABA inhibition of the mitral cells, also permits the formation of a specific olfactory memory without the occurrence of mating. CF: centrifugal fiber of norepinephrine projection from locus ceruleus; E: intracellular effector; G: G protein; $GABA_AR$: $GABA_A$ receptor; αAR: α-adrenergic receptor; VN: vomeronasal nerve. The signaling pathways that inhibit GABA release are marked by minus symbols. (From Kaba et al. 1994).

Thus, the recent progress in the study of learning and memory in higher vertebrates is remarkable. If we assume that similar mechanisms are also involved in the olfactory imprinting in the salmon, we may get some hints from these studies for the study of salmon homing. For example, a research plan to answer the following questions seems to be interesting. (1) Are there any evidences for Hebbian type learning process in the fish olfactory system? (2) If so, which types of neurotransmitter receptors (NMDA or non-NMDA type) are involved? (3) Do any hormones, such as the thyroid hormone, have influences on the learning process? Researches addressing these questions are now in progress in our laboratory, although no data will be presented here.

Another important approach to the study of olfactory mechanism of salmon homing may be to record and analyze the neural activity from animals actually selecting the home stream during the spawning migration. Therefore, we have developed a wired- as well as a nonwired-telemetry systems for transmitting olfactory bulbar EEGs under freely behaving condition. We used the himé salmon (landlocked sockeye salmon, *Oncorhynchus nerka*; known as kokanee in North America), which migrate in Lake Chuzenji. We also used the non-migratory rainbow trout (*Oncorhynchus mykiss*) as experimental fish.

Figure 3 shows a scene from the wired telemetry experiments. A recording electrode was chronically implanted into the olfactory bulb of the himé salmon, which freely swam in the experimental aquarium.

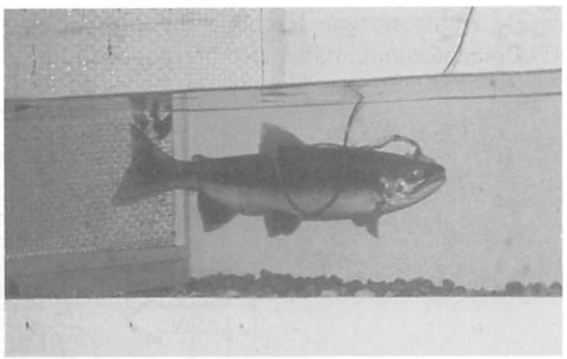

Figure 3. Himé salmon photographed in a wired telemetry experiment. Recording electrode was chronically implanted into the olfactory bulb of a freely swimming himé salmon, and was connected to the preamplifier by way of a lead wire and Hg-Pt sliding contact.

Waters from 4 different streams including the presumed home stream for the migrating himé salmon were pumped, and passed through the experimental aquarium. Figure 4 illustrates the map of 4 collecting sites of the test waters. The number shows the collecting sites, while the star shows the site of experimental aquarium. In this experiment we used the himé salmon which returned to the watercourse from the Lake Chuzenji Fishery Association (CFA), the hatchery of the himé salmon. So, the water from CFA watercourse seems to be the home water for our test fish.

Figure 5 shows an example of the EEG response from the himé salmon to the presumed home stream water (CFA watercourse water). A relatively large EEG

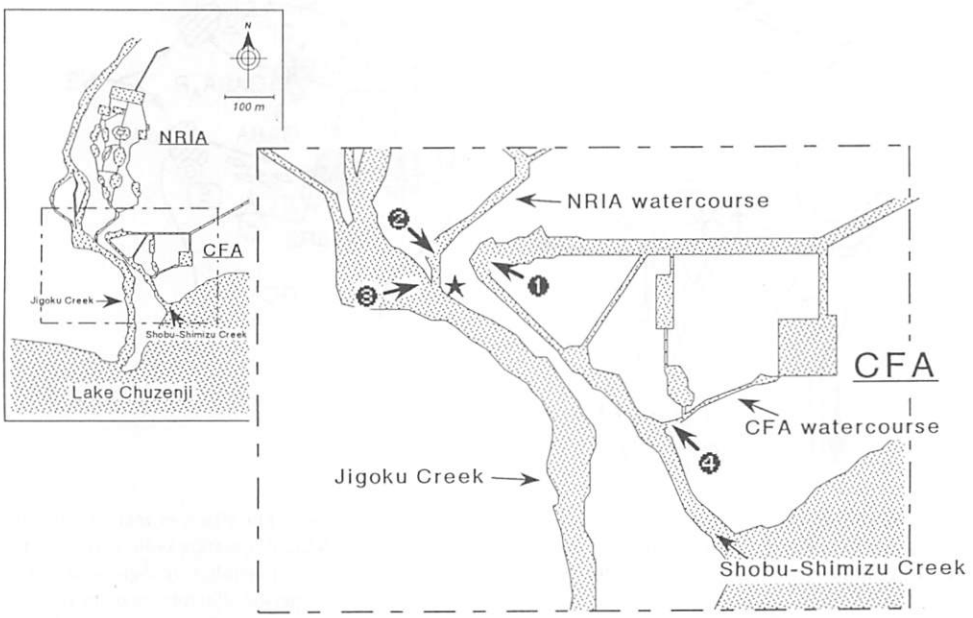

Figure 4. Collecting sites of test waters. The number shows the collecting sites, while the star shows the site of experimental aquarium.

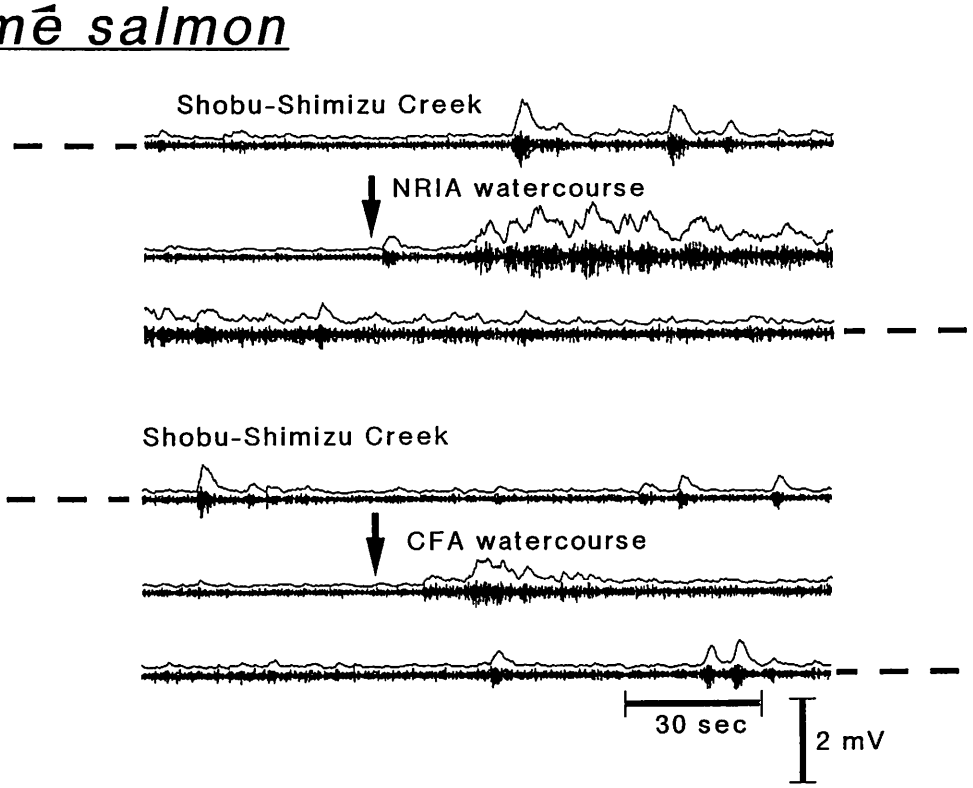

Figure 5. Olfactory bulbar EEG responses to stream waters. CFA watercourse is the presumed home stream for this salmon. Note that the response to NRIA watercourse water (non-home stream water) was larger than that to the home stream water.

response to the home stream water was observed. It should be noted that a spontaneous EEG activity was observed with a considerable magnitude for the adapting water (the Shobu-Shimizu Creek water, in this case). This was never observed in experiments performed under the acute, immobilized condition. However, even a larger EEG response was evoked by a non-home stream water, that is, by the water from the NRIA (National Research Institute of Aquaculture) watercourse (Figure 5).

This suggests that the magnitude of the olfactory bulbar EEG response does not reflect the memory for the home stream imprinted, but rather reflects the strength of odors contained. Presumably, the waters from the CFA and NRIA watercourses contained strong odors, such as the odors from other fish held in the ponds, or the odors from the fish food. However, there remains a possibility that the olfactory bulbar EEG response still contains some components of the home stream memory imprinted. Similar results were obtained from the non-migratory rainbow trout (not illustrated).

To record the EEG response from the fish under a more natural condition, that is, from the fish freely swimming in natural environments, we have developed an underwater radiotelemetry system (Figure 6; Satou et al. 1993; Kudo et al. submitted a,b). The transmitter used 1.6 kHz-PFM (pulse frequency modulation) as the subcarrier and 95.2 MHz-FM as the main carrier. The underwater reception antenna that fed signals to the FM receiver was newly designed. Figure 7 shows the photograph of the transmitter and its batteries. The circuit diagram of the transmitter is shown in Figure 8, while the specifications of it are shown in Table 1.

The underwater antenna (which we named as "aquaerial") consisted of an array of component units of

Table 1. Specifications of transmitter

Frequency mode	PFM-FM
Main carrier frequency	95.2 MHz
Subcarrier pulse frequency	1.6 kHz
Subcarrier modulation ratio	50 Hz/mV
Total power consumption	4.8 mW
Supply voltage	± 3 V
Frequency range	1–40 Hz
Input impedance	5 MΩ
Gain of input amplifier	26 dB
Maximum input voltage	2 mV
Dimensions (transmitter package)	10 mm Φ × 26 mm length
Weight in air	4.4 g
Weight in water	1.5 g
Waterproof	Epoxy molded

UNDERWATER RADIOTELEMETRY SYSTEM

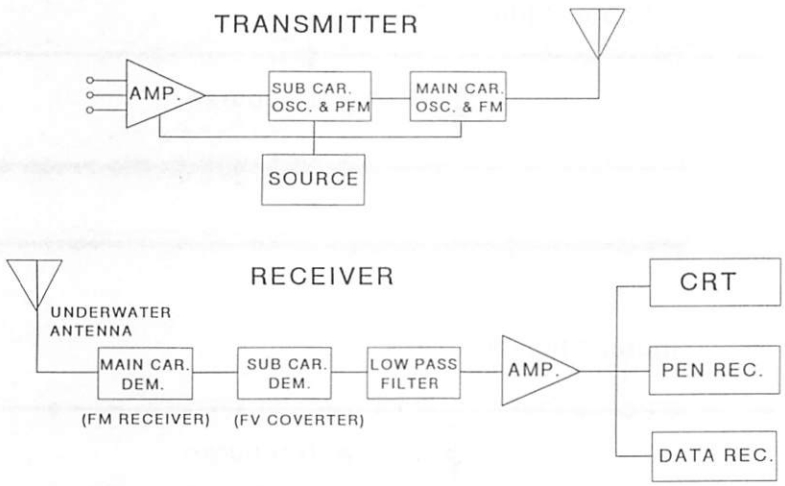

Figure 6. Diagram of the underwater radiotelemetry system.

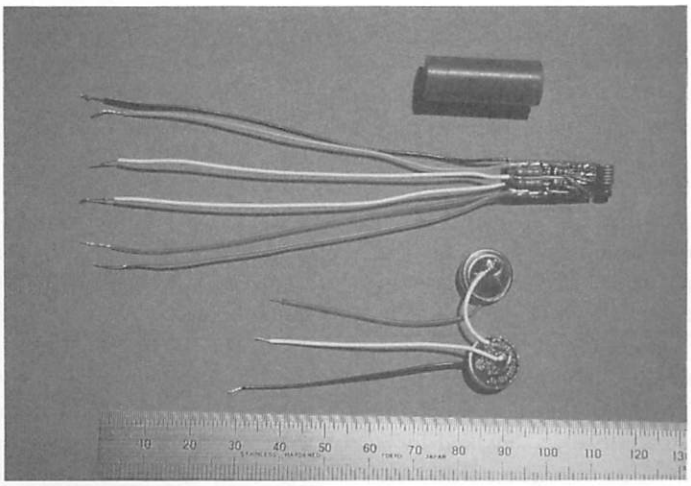

Figure 7. Transmitter and its batteries.

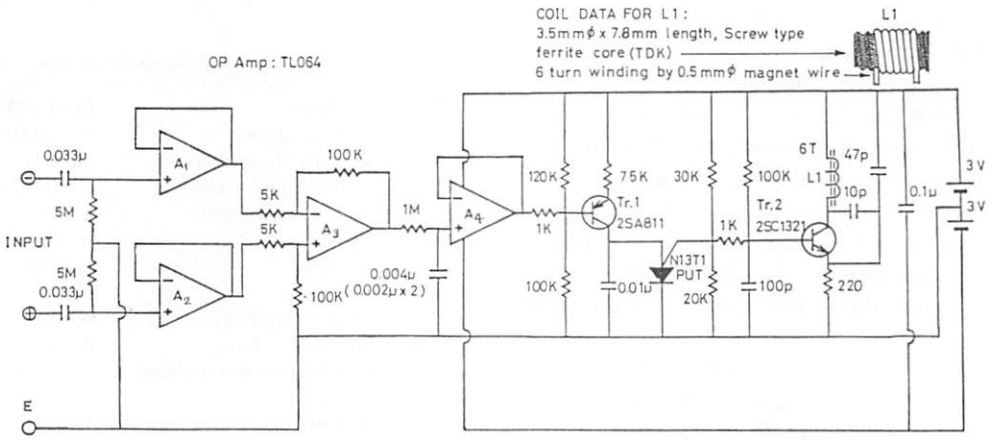

Figure 8. Circuit diagram of the transmitter.

the reception antenna, each of which was connected by a coaxial cable by way of the amplifier circuits (Figure 9). The length of the component antenna was 16.5 cm (1/2 λ of 100 MHz carrier wave in the water, which corresponded to 1/18 λ of the carrier wave in the air), while the interval of the antenna array was 2 m (λ in the coaxial cable, which corresponded to 2/3 λ in the air). Transmission test of the olfactory bulbar EEG signals was performed at the lower part of the NRIA watercourse (Figure 4). The underwater antenna was placed at the bottom of the watercourse.

Figure 10 shows the results of transmission test of the olfactory bulbar EEG wave from the freely swimming rainbow trout. It can be seen that the EEG activity was successfully picked up by the underwater antenna, even when the fish was about 5 m upstream of its end. If the power supply to the amplifiers of the underwater antenna was turned off, no EEG was transmitted, indicating the effectiveness of the present underwater antenna. The present underwater antenna could pick up the EEG signals from any points of the watercourse along the antenna. Figure 11 shows the spontaneous EEG activity 18–22 hours after the start of recording, indicating that long-term recording is possible by using the present telemetry system. Approximately 2 days recording was possible by the batteries used (CR2032, Sanyo), which determined the duration of recording.

Theoretically, the present underwater antenna can be extended lengthily by serially connecting the component units. Therefore, the present radiotelemetry system

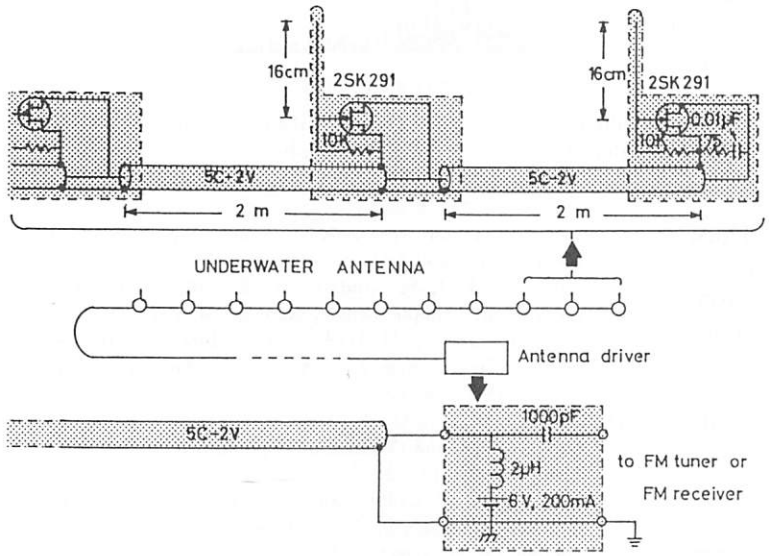

Figure 9. Circuit diagram of the underwater antenna. The underwater antenna consists of an array of component units of reception antenna. Each antenna unit (16 cm long) is connected by the coaxial cable (5C-2V) of 2 m long via the amplifier circuits. The portions of the underwater antenna and the antenna driver indicated by arrows are expanded and illustrated as hatched drawings.

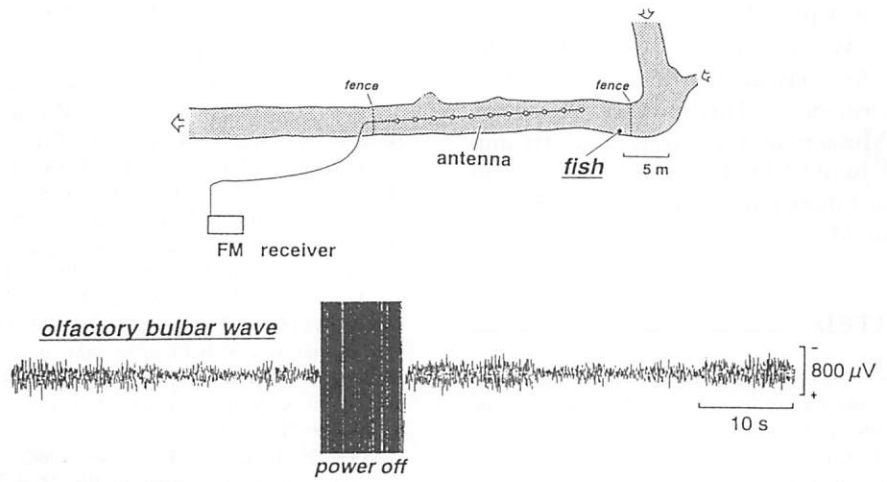

Figure 10. Transmission test of the olfactory bulbar EEG wave. Note that no EEG was transmitted, if the power supply to the amplifiers of the underwater antenna was turned off, indicating the effectiveness of the present underwater antenna.

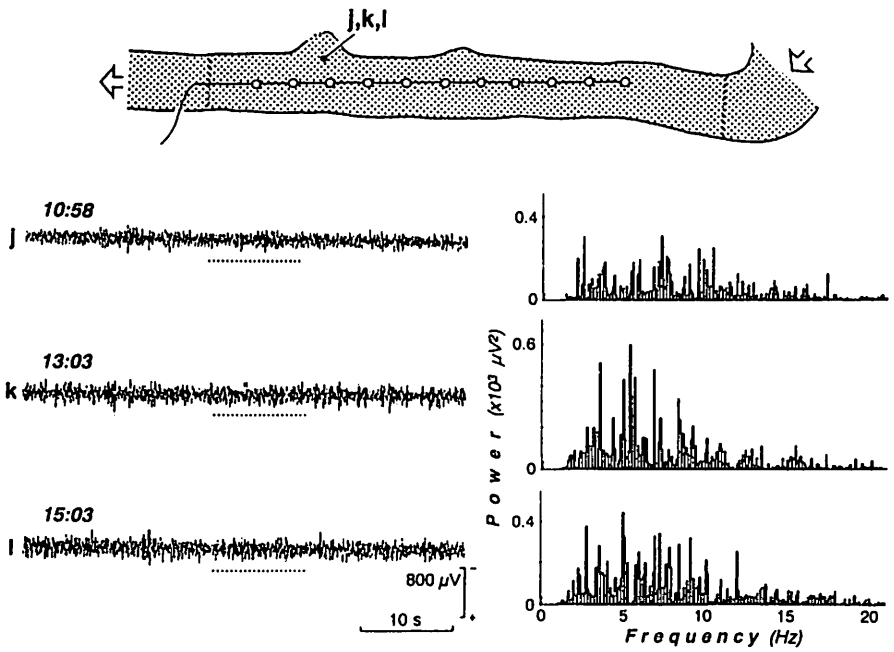

Figure 11. Spontaneous EEG activities 18–22 hours after the start of recording. Graphs in the right column are power spectra during the periods indicated by dotted lines under the EEG records.

seems to be applicable to a longer river system. Thus, we now have an effective underwater radiotelemetry system for transmitting the olfactory EEG activity from freely behaving salmonid fish in the natural environments.

We are planning to record the olfactory EEG activity from the migrating himé salmon which is actually selecting the home stream.

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LITERATURE CITED

Bertmer, G., and R. Toft. 1969. Sensory mechanisms of homing in salmonid fish I. Introductory experiments on the olfactory sense in grilse of Baltic salmon (*Salmo salar*). Behaviour 35: 235–241.

Bliss, T. V. P. and G. L. Collingridge. 1993. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361: 31–39.

Bodznick, D. 1975. The relationship of the olfactory EEG evoked by naturally-occurring stream waters to the homing behavior of sockeye salmon (*Oncorhynchus nerka* (Walbaum)). Comp. Biochem. Physiol. 52A: 487–495.

Brennan, P., H. Kaba, and E. B. Keverne. 1990. Olfactory recognition: a simple memory system. Science 250: 1223–1226.

Cooper, J. C., and A. D. Hasler. 1974. Electroencephalographic evidence for retention of olfactory cues in homing coho salmon. Science 183: 336–338.

Donaldson, L. R., and G. H. Allen. 1957. Return of silver salmon, *Oncorhynchus kisutch* (Walbaum) to point of release. Trans. Am. Fish. Soc. 87: 13–22.

Groves, A. B., G. B. Collins, and P. S. Trefethen. 1968. Roles of olfaction and vision in choice of spawning site by homing adult chinook salmon (*Oncorhynchus tshawytscha*). J. Fish. Res. Board Can. 25: 867–876.

Hara, T. J. 1970. An electrophysiological basis for olfactory discrimination in homing salmon: a review. J. Fish. Res. Board Can. 27: 565–586.

Hara, T. J., and S. B. Brown. 1979. Olfactory bulbar electrical responses of rainbow trout (*Salmo gairdneri*) exposed to morpholine during smoltification. J. Fish. Res. Board Can. 36: 1186–1190.

Hara, T. J., and S. MacDonald. 1975. Morpholine as olfactory stimulus in fish. Science 187: 81–82.

Hara, T. J., K. Ueda, and A. Gorbman. 1965. Electroencephalographic studies of homing salmon. Science 149: 884–885.

Harden Jones, F. R. 1968. Fish migration. Arnold, London.

Hasler, A. D., and A. T. Scholz. 1983. Olfactory imprinting and homing in salmon. Springer-Verlag, Berlin, New York.

Hasler, A. D., A. T. Scholz, and R. M. Horrall. 1978. Olfactory imprinting and homing in salmon. Amer. Sci. 66: 347–355.

Hasler, A. D., and W. J. Wisby. 1951. Discrimination of stream odors by fishes and its relation to parent stream behavior. Amer. Natur. 85: 223–238.

Hassler, T. J. and S. T. Kucas. 1988. Returns of morpholine-imprinted coho salmon to the Mad River, California. North Amer. J. Fish. Manag. 8: 356–358.

Hayashi, Y., A. Momiyama, T. Takahashi, H. Ohishi, R. Ogawa-Meguro, R. Shigemoto, N. Mizuno, and S. Nakanishi. 1993.

Role of a metabotropic glutamate receptor in synaptic modulation in the accessory olfactory bulb. Nature 366: 687–690.

Hiyama, Y., T. Taniuchi, K. Suyama, K. Ishioka, R. Sato, T. Kajihara, and T. Maiwa. 1967. A preliminary experiment on the return of tagged chum salmon to the Otsuchi river, Japan. Bull. Jap. Soc. Sci. Fish. 33: 18–19.

Jensen, A. L., and R. N. Duncan. 1971. Homing of transplanted coho salmon. Prog. Fish Cult. 33: 216–218.

Johnsen, P. B., and A. D. Hasler. 1980. The use of chemical cues in the upstream migration of coho salmon, *Oncorhynchus kisutch* (Walbaum). J. Fish Biol. 17: 67–73.

Kaba, H., Y. Hayashi, T. Higuchi, and S. Nakanishi. 1994. Induction of an olfactory memory by the activation of a metabotropic glutamate receptor. Science 265: 262–264.

Kaji, S., M. Satou, Y. Kudo, K. Ueda, and A. Gorbman. 1975. Spectral analysis of olfactory responses of adult spawning chum salmon (*Oncorhynchus keta*) to stream waters. Comp. Biochem. Physiol. 51A: 711–716.

Kandel, E. R., and R. D. Hawkins. 1992. The biological basis of learning and individuality. Sci. Amer. September 1992: 53–60.

Kandel, E. R., and T. J. O'dell. 1992. Are adult learning mechanisms also used for development? Science 258: 243–245.

Kudo, Y., M. Satou, Y. Takeuchi, S. Kitamura, and M. Iwata (submitted, a) A simple underwater radio-telemetry system and its application for measuring electroencephalographic activity from the himé salmon (landlocked sockeye salmon, *Oncorhynchus nerka*) freely swimming in outdoor pond.

Kudo, Y., M. Satou, Y. Takeuchi, S. Kitamura, and M. Iwata (submitted, b) A newly designed underwater antenna and its application to underwater radio-telemetry for measuring electroencephalographic activity from the rainbow trout (*Oncorhynchus mykiss*) freely swimming in natural environments.

Lorz, H. W., and T. G. Northcote. 1965. Factors affecting stream location, and timing and intensity of entry by spawning kokanee (*Oncorhynchus nerka*) into an inlet of Nicola Lake, British Columbia. J. Fish. Res. Board Can. 22: 665–687.

Nevitt, G. A., A. H. Dittman, T. P. Quinn, and W. J. Jr. Moody. 1994. Evidence for a peripheral olfactory memory in imprinted salmon. Proc. Natl. Acad. Sci. USA 91: 4288–4292.

Peck, J. W. 1970. Straying and reproduction of coho salmon, *Oncorhynchus kisutch*, planted in a Lake Superior tributary. Trans. Amer. Fish. Soc. 99: 591–595.

Quinn, T. P. 1990. Current controversies in the study of salmon homing. Ethol. Ecol. Evol. 2: 49–63.

Satou, M., Y. Kudo, S. Kitamura, and M. Iwata. 1993. Underwater radiotelemetering of electroencephalographic wave from olfactory bulb of rainbow trout freely swimming in natural river. Neurosci. Res. Suppl. 18: 214.

Scholz, A. T., R. M. Horrall, J. C. Cooper, and A. D. Hasler. 1976. Imprinting to chemical cues: the basis for home stream selection in salmon. Science 192: 1247–1249.

Shatz, C. L. 1992. The developing brain. Sci. Amer. September 1992: 35–41.

Shirahata, S. 1969. Homing of himé salmon–VI: roles of vision and olfaction in home stream detection. Abstr. Ann. Meet. Jap. Soc. Fish.: 44–45 (abstract in Japanese).

Thorpe, J. E. 1988. Salmon migration. Sci. Progr. Oxf. 72: 345–370.

Ueda, K., T. J. Hara, and A. Gorbman. 1967. Electroencephalographic studies on olfactory discrimination in adult spawning salmon. Comp. Biochem. Physiol. 21: 133–143.

Ueda, K., T. J. Hara, M. Satou, and S. Kaji. 1971. Electrophysiological studies of olfactory discrimination of natural waters by himé salmon, a land-locked Pacific salmon, *Oncorhynchus nerka*. J. Fac. Sci. Univ. Tokyo, Sec. IV 12: 167–182.

Wisby, W. J., and A. D. Hasler. 1954. The effect of olfactory occlusion on migrating silver salmon (*O. kisutch*). J. Fish. Res. Board Can. 11: 472–478.

Bull. Natl. Res. Inst. Aquacult., Suppl. 2: 59-64 (1996)

SEQUENTIAL IMPRINTING IN CHINOOK SALMON: IS IT ESSENTIAL FOR HOMING FIDELITY?

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ABSTRACT

Sequential imprinting is a hypothesis based on the concept that a specific series of experience events occur primarily during the period of seaward migration in salmonid smolts. Successful homing is accomplished by returning adults when, during their final migratory phases, they experience these same events in a sequentially reversed order. Final events experienced by smolts during imprinting become initial events experienced by adults during natal stream homing. Evidence from homing studies in chinook salmon from some southeast Alaska hatcheries suggests that imprinting may be flawed or incomplete when smolts are released directly into saltwater without a downstream migratory experience. Even when chinook salmon are raised from fry to smolt stage in a freshwater source common to the marine estuary where fully transformed smolts are released, the lack of a downstream migration experience may, in some fish, circumvent part of the highly evolved and complex imprinting process.

During an 11-yr period (1983-1993), 51 adult strays from four hatcheries (Hidden Falls, Little Port Walter, Port Armstrong, Snettisham) were recovered in Farragut River, a regional stream with an endemic run of chinook salmon. These hatcheries, located 120-200 km from Farragut River, all released smolts directly into estuaries. However, no strays were recovered in Farragut River from another hatchery (Crystal Lake) located only 50 km from this stream. Chinook salmon smolts at Crystal Lake hatchery are released into Crystal Creek a short distance before it flows into the estuary. Does the short downstream migration experience of smolts released into Crystal Creek, according to the sequential imprinting hypothesis, provide more complete imprinting than smolts released directly into estuaries at the other hatcheries? A proposed experimental approach to examine this question and other related issues is discussed.

INTRODUCTION

Precise knowledge of how and when the imprinting process in juvenile anadromous salmon occurs is poorly understood. This process, one of the biological marvels of salmonids, enables adults to return to natal areas with a relatively high degree of accuracy. Some studies have suggested that imprinting occurs in a short time interval, generally associated with the physiological changes during smoltification and downstream migration (Donaldson and Allen 1957; Jensen and Duncan 1971). Other studies, however, suggest that some aspects of imprinting occur at other life stages, perhaps even as preemergent alevins in natal gravels (Brannon 1982; Courtenay 1989; Quinn and Dittman 1992). Details of imprinting are to likely vary across species and different life histories. There is little doubt that imprinting is a highly complex process dependent on a chain of interrelated events during juvenile stages. Importance of these events may also vary in accordance with environmental and physiological conditions, for example, hormone levels during critical periods.

The accuracy of homing, influenced by imprinting, thus becomes dependent on many interrelated factors that occur both during juvenile life history stages and during the terminal migratory phase of adults. These factors may include the level or degree of imprinting during juvenile stages (i.e., partial imprinting), environmental changes in the natal freshwater areas during the ocean sojourn, and a genetic tendency for some individuals in some populations to stray (Larkin 1981; Quinn 1984; Leider 1989; Pascual and Quinn 1994; Thorpe 1994). Quinn (1993) examined the question of differential homing and straying among wild and hatchery produced salmon. He considered many issues that influence homing behavior and found that the prevalence of straying varied greatly among populations. Evidence that standard hatchery practices increased straying rates was equivocal, but it was clear that certain hatchery practices could increase

straying (Quinn 1993).

Harden Jones (1968) first outlined in detail the hypothesis of sequential imprinting where the homing mechanism in adult salmon depends on receiving a series of stimuli in the reverse sequential order of that imprinted in young fish. A downstream migratory experience in juveniles was implicit in this concept and in adults a "..... matched imprint facilitates, but an unmatched imprint blocks access to the next in the [reversed sequence] series" (Harden Jones 1968). Several studies have since shown that sequential stimuli, received in the proper order, are necessary for successful homing in salmonids (Gunnerod et al. 1988; Heggberget et al. 1991; Solazzi et al. 1991; Hvidsten et al. 1994).

The present study will examine a data series on homing and straying of chinook salmon, *Oncorhynchus tshawytscha*, from southeast Alaska hatcheries that raise certain questions relative to the principle of sequential imprinting, some hatchery practices, and a stream that has a unique, non-random attraction for hatchery strays.

HATCHERY STRAYS IN SOUTHEAST ALASKA

Chinook salmon resources in southeast Alaska consist of 35 natural spawning populations and 14 hatcheries that culture and release some juveniles of this species. Many of the spawning populations are in remote, often glaciated, streams that have only a few hundred spawners annually. Marine waters in the area contain a complex mixture of chinook salmon stocks originating within the southeast Alaska region and from streams and hatcheries from more southerly, non-Alaska sources. The region is composed of large and small islands along a narrow mainland of coastal fjiords and mountains. With few exceptions, natural spawning chinook salmon populations are located in mainland streams and hatcheries that raise this species are located on islands.

Development of hatcheries and enhancement programs for chinook salmon in southeast Alaska, begun in the mid-1970s, was designed to minimize hatchery-wild stock interactions. Considerations were taken into account for siting of facilities, broodstock selection, fish health issues, and genetic protocols. The primary purpose of these programs was to provide more Alaska-origin chinook salmon for local fisheries and to reduce fishing mortality on depressed wild stocks. Between 1979 and 1993, over 40,000 adult chinook salmon were examined on natural spawning grounds in southeast Alaska to look for, among other things, the presence of hatchery fish marked with coded wire tags. Fifty-six tagged chinook salmon from regional hatcheries were recovered in wild systems representing, when expanded for tagging ratios, 123 fish, 0.3% of adults examined (Heard et al. 1995).

STRAYING INTO FARRAGUT RIVER

While overall straying of hatchery-origin chinook salmon into streams with natural runs was low, in one stream, Farragut River, a glacial stream on the mainland of central southeast Alaska, hatchery strays represented 8.3% of the 616 chinook salmon examined during an 11-yr period, 1983–1993. Moreover hatchery strays into the Farragut River were all from a small group of only four hatcheries (Table 1). Three of these hatcheries, Little Port Walter, Hidden Falls, and Armstrong-Keta, are located on the east side of Baranof Island; the fourth hatchery, Snettisham, is located on the mainland north of Farragut River. All four hatcheries are 120 to 200 km distant from Farragut River. A fifth hatchery, Crystal Lake, with no recorded strays into Farragut River is located on Mitkof Island about 50 km from the stream (Figure 1). These observations have lead to the obvious paradoxical

Table 1. Summary of Farragut River chinook salmon surveys, 1983–1993, and recoveries of hatchery-origin coded-wire tagged adult strays

Year	Adults Sampled (N)	Coded-wire tagged adults collected by hatchery and, in parentheses, expanded number of fish these tags represent.[1]					
		LPW	HF	AK	SN	CL	Totals
1983	59	—	—	—	—	—	0
1984	79	3 (3)	—	—	—	—	3 (3)
1985	84	—	—	—	—	—	0
1989	123	3 (3)	—	—	—	—	3 (3)
1991	96	7 (7)	2 (3)	—	—	—	9 (10)
1992	95	12 (13)	1 (11)	1 (3)	—	—	14 (27)
1993	80	4 (4)	—	—	1 (4)	—	5 (8)
	616	29 (30)	3 (14)	1 (3)	1 (4)	—	34 (51)

[1] Expansions of collected tags based on percentages of hatchery fish tagged. LPW, Little Port Walter; HF, Hidden Falls; AK, Armstrong-Keta; SN, Snettisham; CL, Crystal Lake.

question. Why are adults from the four more distant hatcheries straying into Farragut River, while no adults have been observed straying into this stream from the much closer hatchery at Crystal Lake?

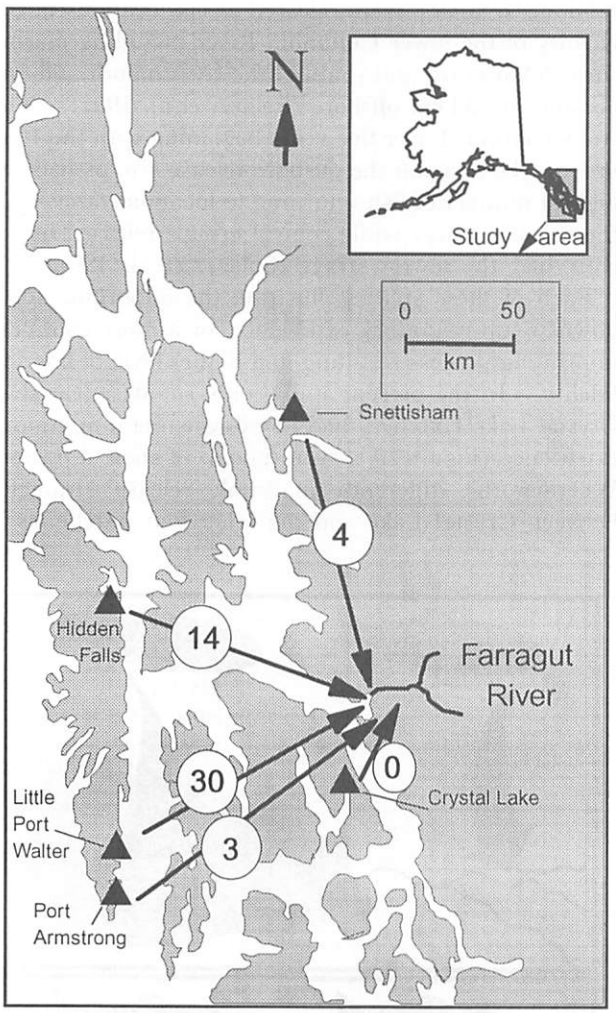

Figure 1. Location of five southeast Alaska chinook salmon hatcheries and Farragut River discussed in this report. Arrows and numbered inserts indicate the expanded number of adult chinook salmon recovered in Farragut River from each hatchery.

The five hatcheries have a number of fish culture practices in common. All are located at or near tidewater on streams or water sources that do not have natural chinook salmon runs. Each hatchery primarily raises age-1 smolts, mostly in freshwater raceways, and smolts from all of the hatcheries are released in mid-May, the nominal migration timing of wild smolts in the region (Meehan and Sniff 1962). While there has been some overwinter netpen culture of age-1 smolts in estuaries at Hidden Falls and Little Port Walter, this hatchery practice does not appear to affect straying into Farragut River. Freshwater culture at all five hatcheries utilizes water from drainages that have lakes near the headwaters of the watershed.

Four different stocks, derived from natural runs of chinook salmon in the region, are raised in the five hatcheries. Andrew Creek stock is raised at Hidden Falls, Armstrong-Keta, Snettisham, and Crystal Lake hatcheries while Unuk River, Chickamin River, and King Salmon River stocks are raised at the Little Port Walter facility. Unuk River stock was also raised for a while at Armstrong-Keta hatchery. The three stocks raised at Little Port Walter are maintained separately throughout the hatchery period. All smolts released at this research station are coded wire tagged by stock and all adults are decoded before spawning. Adult strays from each of these four hatchery stocks of chinook salmon have been recovered in Farragut River and there does not appear to be any stock influence in the propensity for straying into this stream.

One distinctively different hatchery practice that may influence differential imprinting and homing behavior among the five hatcheries was identified. The four hatcheries with documented straying into Farragut River each routinely release smolts directly into the estuary at the hatchery site while Crystal Lake Hatchery releases smolts into Crystal Creek a short distance upstream from where this stream enters its estuary (Table 2). Considering again Harden Jones' principle of sequential imprinting during the downstream migration of smolts, is it possible this difference in smolt

Table 2. Comparison of chinook salmon programs at five southeast Alaska hatcheries, and recoveries of adult strays in Farragut River

Hatchery[1]	Stocks[2]	Timing/Total Smolts Released 1986–1988 (In Millions)	Distance from Farragut River (km)	Principal Culture Methods	Smolt Release Procedures
Four hatcheries with Farragut R. adult recoveries (LPW; HF; AK; SN)	UN CH KSR AC	mid-May; 5.1	120–200	Age-1 smolts; freshwater raceways; some marine net pens	Release into estuary
One hatchery with no recorded recoveries (CL)	AC	mid-May; 3.7	50	Age-1 smolts freshwater raceways	Release into stream

[1] LPW, Little Port Walter; HF, Hidden Falls; AK, Armstrong-Keta; SN, Snettisham; CL, Crystal Lake
[2] UN, Unuk; CH, Chickamin; KSR, King Salmon River; AC, Andrew Creek

release pattens at these five hatcheries account for the observed straying behavior into Farragut River? Does the short downstream migration experience of smolts at Crystal Lake Hatchery provide a more complete imprinting sequence in contrast to smolts released directly into estuaries at the other four hatcheries?

DISCUSSION

In general, documented straying of hatchery chinook salmon into streams with endemic runs from southeast Alaska facilities is low, averaging only 0.3% of adults examined in wild systems during the period, 1979–1993 (Heard et al. 1995). Actual rates are undoubtedly somewhat higher due to the remoteness and difficulty of surveying most wild chinook salmon populations in the region. Nevertheless, with overall documented rates relatively low, the known straying patterns of adults into Farragut River, and especially the lack of strays from nearby Crystal Lake Hatchery raise questions on specific hatchery practices and also on why this river is attractive to strays from certain hatcheries.

The significance of a downstream migration experience relative to homing fidelity can be seen in a number of recent studies. Hansen et al. (1989), examining the role of sequential imprinting, concluded that Atlantic salmon, *Salmo salar*, "..... deprived of their smolt migration did not return to the river to which they were imprinted" and ".... need to experience the outward smolt migration in order to detect their home river". In an earlier study, Hansen et al. (1987) studied migration behavior of Isma River Atlantic salmon in Norway where 4-yr-old farmed fish allowed to escape at sea 4 km and 90 km from Isma River were compared with anosmic-treated farmed fish released 90 km from the river and with 3-yr-old smolts released in the natal stream. They found no fidelity in ability of farmed fish, either anosmic or with olfaction intact, to home to the natal stream regardless of release location, while smolts released into the stream homed with high precision.

Gunnerod et al. (1988) in an effort to improve low returns of hatchery-reared Atlantic salmon smolts made experimental releases of 2-yr-old smolts, over an 8-yr period, in Surna River 20 km upstream from the mouth, in the fjord near the mouth of the Surna and at sea 100 km north of the estuary. In all years, results showed higher recovery rates of sea releases followed by fjord and stream releases, a pattern presumably caused by reduced predator losses of smolts released at sea over smolts released in the river. While significant numbers of sea-released fish did home to Surna River, nearly as many strayed to other rivers. In contrast, all smolts released in Surna River homed to that stream. In a follow-up study on the same river using a similar release pattern, Heggberget et al. (1991) also found increased straying rates of adults when smolts were released at sea or in the estuary over that of smolts released in the river.

In another study designed to examine variations in marine survival of hatchery smolts, yearling coho salmon, *O. kisutch*, were released at six locations in the vicinity of the lower Columbia River including marine sites 19 km north and south of the river mouth and one oceanic site 38 km offshore (Solazzi et al. 1991). The study continued over five years beginning with the 1981 brood. Here again the offshore release groups had the highest number of fish returning to locations other than the natal hatchery while control groups released in the river had the fewest strays (Solazzi et al. 1991).

Each of these studies illustrate the importance of a downstream migratory experience for a more complete imprinting process resulting in a higher level of homing fidelity. In the present study, only smolts released at Crystal Lake Hatchery had any downstream migratory experience (Figure 2A), even though of short duration. Whether the difference in smolt release strategies between Crystal Lake and the other four hatcheries is

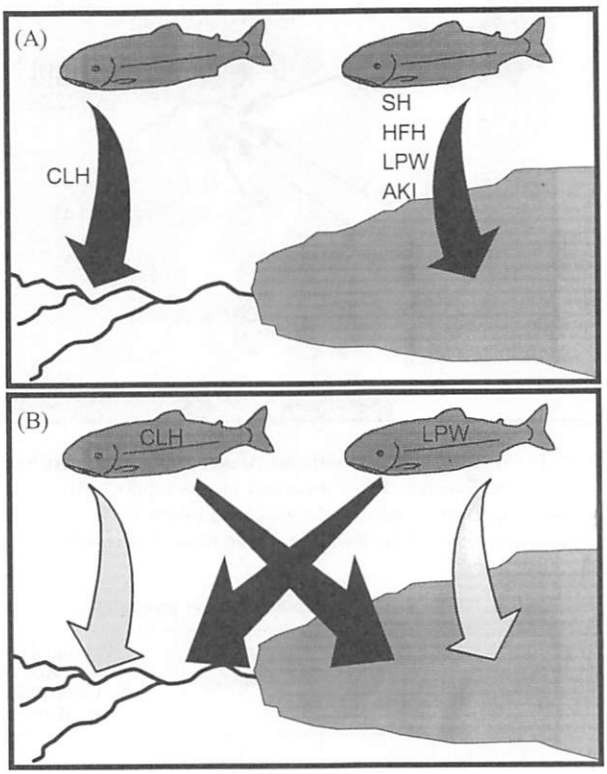

Figure 2. Schematic illustration: (A) of present chinook salmon smolt release practices at five southeast Alaska hatcheries; (B) of proposed reciprocal experiment at two hatcheries to study effects on homing behavior on smolt releases into streams and into estuaries. CLH, Crystal Lake Hatchery; SH, Snettisham Hatchery; HFH, Hidden Falls Hatchery; LPW, Little Port Walter; AKI, Armstrong- Keta Inc.

sufficient to explain the observed straying of hatchery-origin chinook into Farragut River, while perhaps equivocal, still presents a very intriguing issue.

Tagging ratios of released hatchery smolts have a bearing on the future recoveries of tags, illustrated by the greater number of actual tags recovered from Little Port Walter (Table 1) where, with minor exceptions, all smolts are tagged. Other Alaska hatcheries tag only a small portion, generally 20% or less of smolts released. Some Crystal Lake Hatchery adults could also have strayed into Farragut River but no tagged fish were recovered. However, assuming available data do accurately reflect true behavior requires an attempt to explain the paradox. Almost all hatcheries have unique, site-specific factors that set some fish culture practices apart from each other. The common practice of directly releasing smolts into estuaries at Little Port Walter, Hidden Falls, Armstrong-Keta, and Snettisham, with concomitant straying into Farragut River is consistent with the importance of the sequential imprinting principle and the concept of partial or incomplete imprinting.

Partial or incomplete imprinting, however, as a concept, should be considered relative to overall rates of homing or straying for specific populations. From 1981 to 1989, overall homing and straying rates in two hatchery-produced chinook salmon stocks were measured at Little Port Walter. This study involved the release of 1.8 million tagged smolts from 1976 through 1987 broods. Of 22,198 maturing chinook salmon recovered at or near potential spawning areas, 98.8% returned to the natal release site at Little Port Walter. Although one or more strays were documented from 25 different sites, most (64%) were recovered in nearby non-chinook salmon watercourses less than 25 km from the station (Hard and Heard, inpreparation). Only nine strays were recovered from wild chinook salmon systems. From these data, we can see the overall rate of straying was relatively low, averaging only 1.2%. While direct release of smolts into the estuary and lack of a downstream migration experience may have influenced straying rates, the overall adverse effect on homing fidelity was not great.

Another important issue is that straying from Little Port Walter, Hidden Falls, Armstrong-Keta, and Snettisham hatcheries into wild chinook salmon streams is concentrated in Farragut River. While 34 tagged strays from these four hatcheries were found in that river (Table 1), a region- wide inventory from all southeast Alaska hatcheries into wild chinook salmon spawning streams revealed the next greatest number of strays for a given stream was only seven fish in Chilkat River (Heard et al. 1995).

Straying from the four hatcheries into Farragut River is decidedly non-random. Why this stream holds an attraction for hatchery strays is unclear, but it is similar to the attraction for hatchery strays documented in the Lewis and Kalama rivers in the lower Columbia River (Quinn and Fresh 1984: Quinn et al. 1991).

One possible explanation for non-random straying into Farragut River revolves around ocean migration behavior of some smolts released from the five hatcheries discussed in this report (Figure 1). A late winter-early spring commercial troll fishery often harvests each year several hundreds of tagged, maturing-age chinook salmon from these hatcheries in Frederick Sound and Lower Stephens Passage, marine waters immediately adjacent to Farragut River. Since maturing wild Farragut River chinook salmon are presumably in the same waters at the same time, based on known maturation cycles and stream entry patterns, perhaps endemic stock adults returning to their natal stream provide some sort of a decoy effect on partially imprinted hatchery fish, causing them to enter the stream. Unfortunately, due to glaciated water with poor visibility, little definitive information is available on the run size of wild chinook salmon in this stream. It is thought the Farragut River run may average 1,000 or more spawners annually (Bob Zorich, Alaska Department of Fish and Game, personal communication).

Even without direct interaction between maturing wild-stock and hatchery-stock fish in these particular marine waters, the attraction of Farragut River water alone, may be sufficient to cause non-random straying of some hatchery stocks. This point is especially relevant when considering that many southeast Alaska chinook salmon hatcheries, including those on east Baranof Island, are located in non-chinook salmon areas and often have stream flows and water temperature regimes asynchronous to those of wild stocks in the region. Farragut River water may be attractive to hatchery chinook salmon that happen to be in the influence of this stream during the time of final maturation. This could be especially true if hatchery fish are not completely imprinted sequentially to their natal area.

These conjectures are consistent with the available data presented in this report. It is instructive to reemphasize the lack of strays from Crystal Lake Hatchery, which normally, because of its closer proximity to Farragut River, should be expected to have more strays than more distant hatcheries. These observations provide a basis for suggesting future studies, including controlled experiments, to verify or reject the influence of releasing smolts directly into estuaries vs. providing smolts with a downstream migration experience.

Such a reciprocal study could be done at Crystal Lake and Little Port Walter hatcheries according to the diagram illustrated in Figure 2B. Each hatchery

would release sibling groups of marked smolts both directly into respective estuaries (treatment group for Crystal Lake) and into associated streams (treatment group for Little Port Walter). Conventional release practices at each facility would represent controls. A permant fish control and counting weir is situated where Sashin Creek flows into the head of the estuary at Little Port Walter. This weir, which has been used in the past to prevent adult chinook salmon from entering the stream and interacting with endemic runs of other salmon in Sashin Creek, would be used to monitor downstream migration of treatment groups of smolts released above the weir. At Crystal Lake, treatment groups of smolts would be trucked or transferred through flexible piping directly into the estuary. If this study is undertaken, it should include smolt groups released over two or three broods and would involve a commitment of 6 to 10 yr to monitor adult returns, at hatcheries, regional streams, and especially in Farragut River.

ACKNOWLEDGMENTS

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LITERATURE CITED

Brannon, E. L. 1982. Orientation mechanisms of homing salmonids. *In*: E. L. Brannon and E. O. Salo (*eds.*), Proceedings of Salmon and Trout Migration Behavior Symposium, pp. 219–224. Univ. Wash., School Fish., Seattle.

Courtenay, S. C. 1989. Learning: memory of chemosensory stimuli by underyearling coho salmon (*Oncorhynchus kisutch*). Ph. D. thesis. Univ. British Columbia,Vancouver. 231 pp.

Donaldson, L. R. and G. H. Allen. 1957. Return of silver salmon, *Oncorhynchus kisutch* (Walbaum) to point of release. Trans. Am. Fish. Soc. 87: 13–22.

Gunnerød, T. B., N. A. Hvidsten, and T. G. Heggberget. 1988. Open sea releases of Atlantic salmon smolts, *Salmo salar*, in central Norway, 1973–1983. Can. J. Fish. Aquat. Sci. 45: 1340–1345.

Hansen, L. P., K. B. Døving, and B. Jonsson. 1987. Migration of farmed Atlantic salmon with and without olfactory sense released on the Norwegian coast. J. Fish. Biol. 30: 713–721.

Hansen, L. P., B. Jonsson, and R. Andersen. 1989. Salmon ranching experiments in the River Imsa: is homing dependent on sequential imprinting of the smolts? *In*: E. Brannon and B. Jonsson (*eds.*). Proceedings of Salmonid Migration and Distribution Symposium, pp. 19–29. Univ. Wash., School Fish., Seattle. NINA, Trondheim, Norway.

Hard, J. J. and W. R. Heard. Inpreparation. Homing and straying patterns in two stocks of chinook salmon transplanted to Little Port Walter, southeastern Alaska. Natl. Mar. Fish. Serv., Montlake Lab., Seattle.

Harden Jones, F. R. 1968. Fish Migration. Edward Arnold, London.

Heard, W., R. Burkett, F. Thrower, and S. McGee. 1995. A review of chinook salmon resources in southeast Alaska and development of an enhancement program designed for minimal hatchery-wild stock interaction. Am. Fish. Soc. Symp. 15: 21–37.

Heggberget, T. G., N. A. Hvidsten, T. G. Gunnerød, and P. I. Møkkelgjerd. 1991. Distribution of adult recaptures from hatchery-reared Atlantic salmon (*Salmo salar*) smolts released in and off-shore of the River Surna, western Norway. Aquaculture 98: 89–96.

Hvidsten, N. A., T. G. Heggberget, and L. P. Hansen. 1994. Homing and straying of hatchery-reared Atlantic salmon, *Salmo salar* L., released in three rivers in Norway. Aquacult. Fish. Manage. 25(Suppl. 2): 9–16.

Jensen, A. L. and R. N. Duncan. 1971. Homing of transplanted coho salmon. Prog. Fish-Cult. 33: 216–218.

Larkin, P. 1981. A perspective on population genetics and salmon management. Can. J. Fish. Aqua. Sci. 38: 1469–1475.

Leider, S. A. 1989. Increased straying by adult steelhead trout, *Salmo gairdneri*, following the 1980 eruption of Mount St. Helens. Environ. Biol. Fishes 24: 219–229.

Meehan, W. R. and D. B. Siniff. 1962. A study of the downstream migrations of anadromous fishes in the Taku River, Alaska. Trans. Am. Fish. Soc. 91: 399–407.

Pascual, M. A. and T. P. Quinn. 1994. Geographical patterns of fall chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), from Columbia River (USA) hatcheries. Aquacult. Fish. Manage. 25(Suppl. 2): 17–30.

Quinn, T. P. 1984. Homing and straying in Pacific salmon. *In*: D. McCleave, G. P. Arnold, J. J. Dodson, and W. H. Neill. (*eds.*) Mechanisms of Migration in Fishes, pp. 357–362. Plenum Press, New York.

Quinn, T. P. 1993. A review of homing and straying of wild and hatchery-produced salmon. Fish. Res. 18: 29–44.

Quinn, T. P. and K. Fresh. 1984. Homing and straying in chinook salmon (*Oncorhynchus tshawytscha*) from Cowlitz River hatchery, Washington. Can. J. Fish. Aquat. Sci. 41: 1078–1082.

Quinn, T. P., R. S. Nemeth, and D. O. Mc Issac. 1991. Homing and straying patterns of fall chinook salmon in the lower Columbia River. Trans. Am. Fish. Soc. 120: 150–156.

Quinn, T. P. and A. H. Dittman. 1992. Fishes. *In*: F. Papi (ed.) Animal Homing, pp. 145–211. Chapman and Hall Animal Behavior Series, London.

Solazzi, M. F., T. E. Nickelson, and S. L. Johnson. 1991. Survival, contribution and return of hatchery coho salmon (*Oncorhynchus kisutch*) released into freshwater, estuarine, and marine environments. Can. J. Fish. Aquat. Sci. 48, 248–253.

Thorpe, J. E. 1994. Significance of straying in salmonids and implications for ranching. Aquacult. Fish. Manage. 25(Suppl. 2): 183–190.

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A REAL TIME ANALYSIS SYSTEM FOR GENE EXPRESSION IN TRANSGENIC FISH

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ABSTRACT

It takes a long time to select living transgenic fish, because many fish derived from fertilized eggs with the introduced exogenous gene must be kept for a long time until high molecular weight DNA can be extracted from their fins. It is therefore very useful for the selection of transgenic fish to be able to analyze the expression pattern of the introduced gene at early embryonic stages in living embryos. A technique which can analyze the expression pattern of the introduced gene using a firefly luciferase reporter gene is being developed. In living embryos, expression of the luciferase gene could be detected 6 hr after injection.

INTRODUCTION

Recently, molecular biologists have become interested in fish development (Mullins et al. 1993, Concordet and Ingham 1994), especially the establishment of the central nervous system. Many genes involved in fish development have been cloned, and their sequences and molecular characteristics have been analyzed. Since only a limited number of cell lines are available for fish, these genes must be transferred directly into the fish for studies of gene expression and function. Introduction of the gene is usually performed using conventional methods such as microinjection. It is difficult to introduce exogenous genes into the male pro-nucleus in fertilized eggs of fish. The efficiency of gene transfer and integration into chromosomes is usually low using these methods (Westerfield et al. 1992, Lin and Hopkins 1994, Tamiya et al. 1990, Muller et al. 1993, Alestrom et al. 1992). Therefore, it was necessary to develop a technique in which the gene could be efficiently transferred and integrated at early developmental stages. It takes a long time to select living transgenic fish, because many fish derived from fertilized eggs with the introduced exogenous gene must be kept for a long time until high molecular weight DNA can be extracted from their fins. For the selection of transgenic fish, it would be very useful to be able to analyze the expression pattern of the introduced gene at early embryonic stages in living embryos. We are developing a technique which can analyze the expression pattern of the introduced gene, using a firefly luciferase gene as a reporter gene.

MATERIALS AND METHODS

ZEBRAFISH CULTURE AND MICROINJECTION

Zebrafish (*Brachydanio rerio*) were obtained from a pet shop and kept in water at 28°C on a15 hr light/9 hr dark regime. About 50 picoliters (pl) of super-coiled plasmid DNA (50 μg/ml) were injected into recently fertilized embryos prior to, or shortly after, the first cleavage, essentially as described previously (Stuart et al. 1988). Cleavage was delayed by incubation in 3× diluted Ringer solution (Yamamoto 1961) at 17°C after egg collection and during microinjection. The DNA solution contained 0.25% phenol red to aid in the estimation of the injection volume. After microinjection, the eggs were transferred to sterile water and incubated at 28°C in the dark until hatching of the larvae.

PLASMID CONSTRUCTS

The 1.9 kb Hind III/ SmaI fragment from plasmid pRSVL containing the coding sequence of the firefly luciferase gene was subcloned into the Hind III/ EcoRV sites of plasmid pBluescript II KS(+) (Stratagene), excised by digestion with Hind III and BamHI, and the

fragment subsequently cloned into the Hind III/ BamHI sites of plasmid pCEP4 (Invitrogen), yielding plasmid pCEP4-luc (Figure 1).

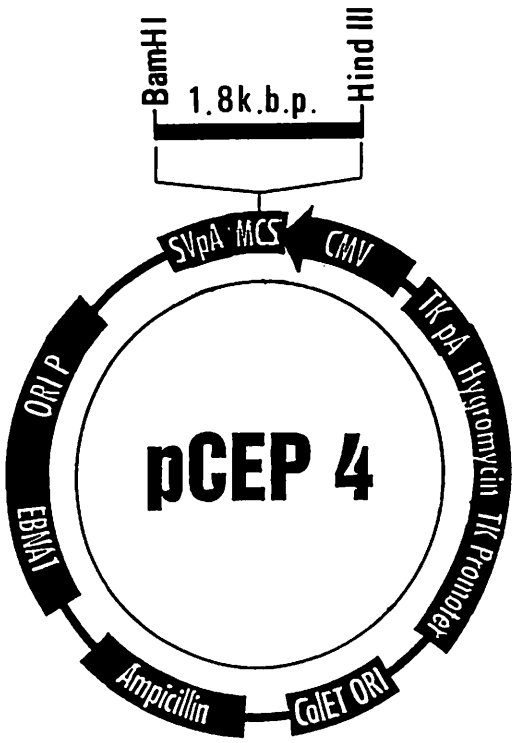

Figure 1. The expression vector, pCEM-luc
The expression vector was constructed having a cytomegalo virus promoter and enhancer, and a firefly luciferase cDNA gene.

Another expression vector was constructed, having a promoter of an amago salmon growth hormone gene and the firefly luciferase cDNA gene. The enhancer and promoter of cytomegalovirus were removed from pCEP4-luc by digestion with Xba I/ Pvu II, and the promoter of amago salmon growth hormone gene was integrated into the site.

SOUTHERN BLOT ANALYSIS OF GENOMIC DNA

Individual fish were collected either 2 or 14 days after hatching and incubated in lysis buffer (0.5% SDS, 400 μg/ml proteinase K, 150 mM NaCl, 50 mM Tris/HCl) at 37°C for 4 hr (Blin et al. 1976). The lysed cells were phenolized, and DNA precipitated with ethanol. The complete DNA of one individual fish, digested with KpnI, was used for separation on a 0,8% agarose gel and subsequent blotting and hybridization procedures (Southern 1975). Plasmid pCEM-luc was used as a probe.

ANALYSIS OF LUCIFERASE EXPRESSION IN VIVO

Light emission from transformed embryos was monitored as early as 6 hr after injection of DNA and during the subsequent stages of development. Transformed eggs or hatched fish were placed on the lid of a microtiter plate and covered with a drop of 0,1mM firefly luciferin in H_2O(Sigma). For screening of up to 96 individuals, the lid was centered in the dark chamber of a low-light video-image analyzer (ARGUS-50, Hamamatsu Photonics, Hamamatsu, Japan) and emitted photons were collected for 20 min. To monitor the spatial distribution of light emission in living fish or embryos, the light sensitive camera was connected to an Olympus IMT-2 microscope and individual transformants were analyzed under 4.0× magnification. Eggs and small larvae were covered with a small piece of Saran Wrap to prevent movement during analysis. Larger fish were additionally anaesthetized with FA100 (Tanabe Yakuhin Co., Japan) before measurement. Photon accumulation was carried out for 1～20 min depending on the individual transformant. Sony UV7100 video printer was used to print the images.

RESULTS

EXPRESSION OF FIREFLY LUCIFERASE IN ZEBRAFISH

Several thousand fertilized zebrafish eggs were injected with the plasmid construct pCEM-luc, carrying the gene for firefly luciferase under the control region of the strong cytomegalovirus. All injections were performed at the one- or two-cell stage of the eggs. Survival rates for injected eggs, and the percentage of hatched, normal looking-larvae were calculated from 1780 specimens and ranged from 16% to 41% (Table 1). Initial screening of the transformed eggs by low-light video-image analysis revealed light emission as early as 6 hr after injection. An earlier onset of luciferase gene expression is possible, but has not yet been investigated.

Table 1. Survival and transformation rates of microinjected zebrafish eggs calculated from 7 independent experiments

Injected eggs	Hatched larvae	Transformed larvae
220	46 (20%)	20 (9%)
180	31 (17%)	12 (6%)
180	30 (16%)	16 (9%)
300	119 (40%)	35 (12%)
300	122 (41%)	41 (14%)
300	68 (23%)	19 (6%)
300	109 (36%)	50 (17%)

To determine the transformation rate, light emissions from two-day-old larvae were measured for 20 min; 6% to 17% of the microinjected individuals expressed the luciferase gene (Table 1). All the transgenic fish were found to be mosaic, and could be classified into one of three general types (Figure 2). In the first type, the luciferase gene was expressed in half of the body. In the second type, the gene was expressed in discrete patches. The last type showed only transient luciferase expression in the yolk sac. After 14 days, gene expression was seen only in the intestines, gills and the tissues near these organs; fry at this stage can only absorb luciferin from intestine and gills (Figure 3).

This observation was confirmed by Southern blot hybridization of genomic fish DNA (figure 4). Genomic DNA of 6 bioluminescent zebrafish were extracted 14 days after hatching. The DNA of another 5 individuals, which initially emitted light in the yolk sack but had subsequently lost this ability, and 3 non-injected control fry, was also extracted 14 days after hatching. The hybridization patterns of all 6 biolu-

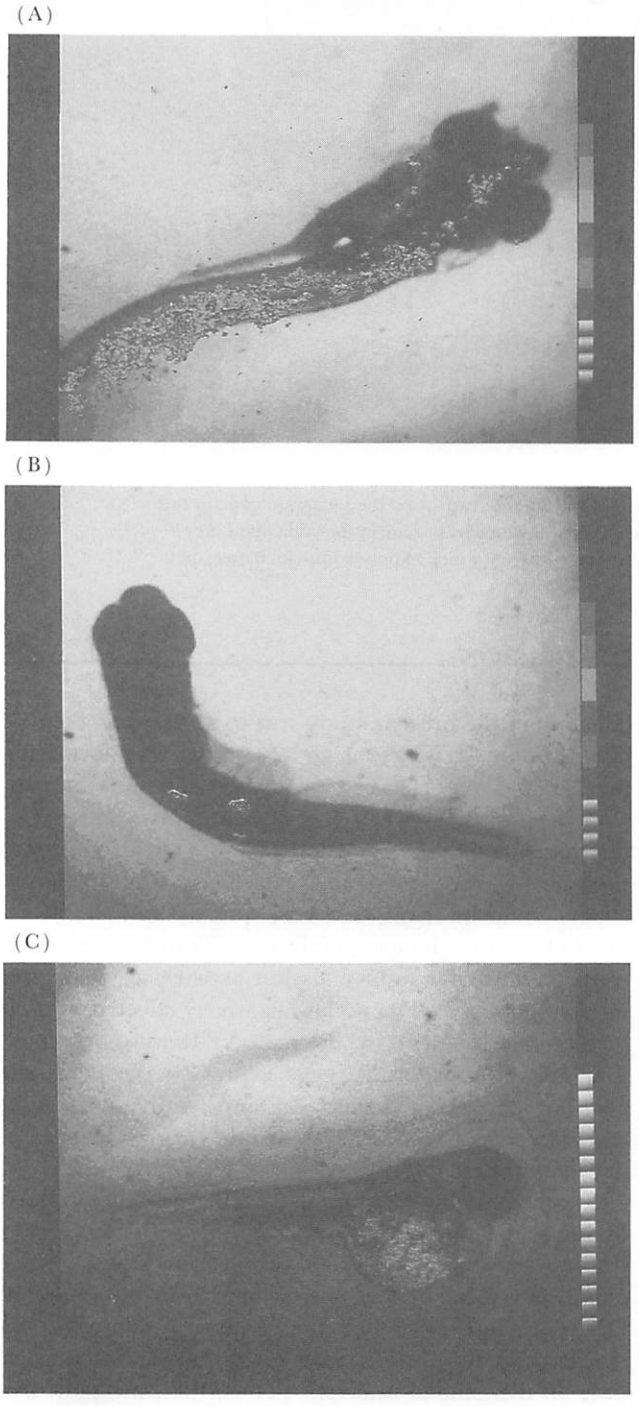

Figure 2. The expression pattern of firefly luciferase gene in 4-day hatched fry.

All transgenic fish were mosaic and were classified into three types. (1) The luciferase gene was expressed in half of the body. (2) The gene was expressed in one part of the body. (3) The luciferase gene was transiently expressed in the yolk sac. When fry absorbed the yolk, the gene was resorbed.

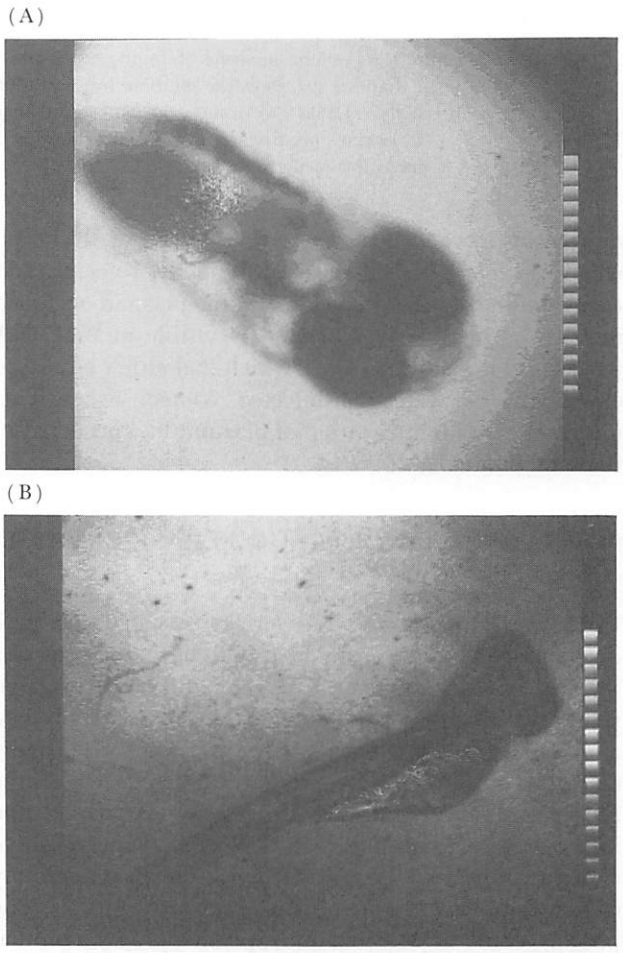

Figure 3. The expression pattern of firefly luciferase gene in 7-day hatched fry.

After 7 days, the expression of the gene was observed only in the intestines, gills and tissues near these organs, since fry at this stage absorb luciferin only from the intestines and gills. (1) The expression pattern of the luciferase gene in and near gills at 7 days. (2) The expression pattern of the gene in and near intestine at 7 days.

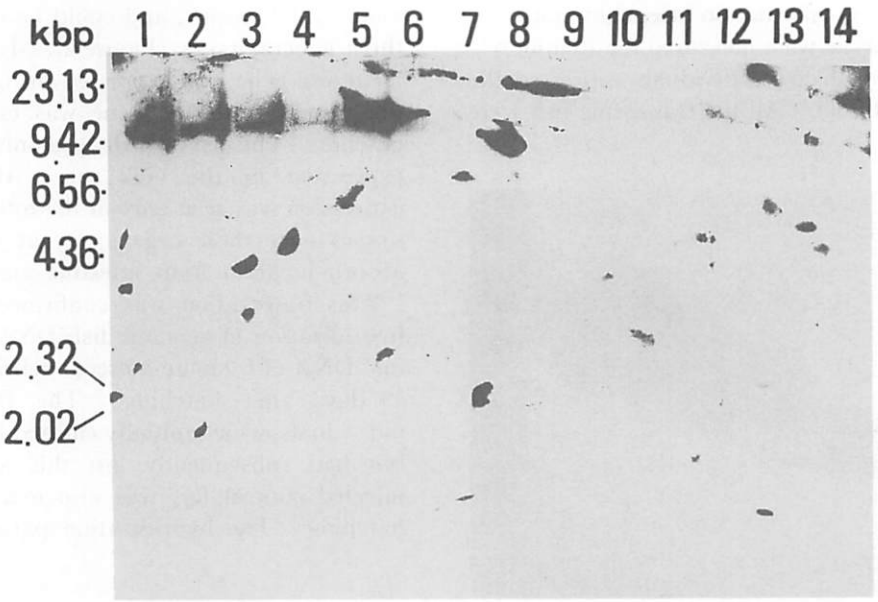

Figure 4. Genome analysis of 14 fry by Southern hybridization.
Lanes 1 to 6 show the southern hybridization patterns of the fry that expressed the luciferase gene in the body. Lanes 7 to 10 show the patterns of the fry that expressed the luciferase gene in the yolk, and lanes 11 to 14 show negative controls. This confirms that at this stage, the fry which expressed the luciferase gene have the luciferase gene.

minescent larvae show one band of about 14 kb.p., which is largert than the size of the pCEP4 vector, and theirfor indicats that integration of the plasmid occurred in these individuals. No band is visible in the DNA patterns of the other 8 fishes, which had either ceased to emit light or were non-injected control fish. This indicates that no integration of plasmid had occurred in these individuals.

SPATIAL IMAGING OF LUCIFERASE EXPRESSION *IN VIVO*

Another expression vector were constructed, having a promoter of an amago salmon growth hormone gene and the firefly luciferase cDNA gene. The expression vector was microinjected into fertilized eggs of zebrafish, and the eggs were incubated at 28°C. The living embryos or hatched fry were transferred to 0.1mM luciferin solution and the expression pattern of the introduced gene was analyzed by the light supersensitive camera connected to a microscope. On the fourth day of development, faint of the gene expression (one photon) could be detected in the pituitary; only a limited amount of luciferin may reach the pituitary and the expression of the growth hormone gene may be very weak at this early stage. We are currently attempting to apply this analysis system to the amago salmon embryo.

DISCUSSION

The aim of the present study was to assess the potential of the firefly luciferase gene an *in vitro* transformation marker and reporter of gene expression in zebrafish.

Gene transfer was accomplished by means of microinjection of the plasmid DNA into fertilized eggs at the one- or two-cell stage. Successful transformation events could be monitored very quickly and simply by low-light video-image analysis. Transformed eggs were screened for light emission as early as 6 hr after microinjection, and were not negatively effected in their further development by the assay. It must be noted that not all transformed eggs expressed the luciferase gene at such early stages. In some individuals, light emission in the yolk sac was not detected even several days after hatching.

Another common feature of transformed fish obtained by microinjection of plasmid DNA into fertilized eggs is the mosaic expression of the exogenous DNA. Since one or several cell divisions occured before the foreign DNA was integrated into the genome of one cell, only the descendants of this particular cell would inherit and pass on the gene of interest. Thus, the transgenic fish would express the reporter gene only in the tissues that derived from these cells. In the case of non-integrated DNA, the plasmid number per cell wiould be reduced with every cell division until the plasmid would ultimately be lost altogether unless the plasmid replicated autonomously. Firefly luciferase and β-

galactosidase expression has been found in the yolk sphere of transformed catfish eggs (Mullef et al. 1993), suggesting a movement of transgene product into the vegetal pole of the egg.

Screening for transformed embryos and larvae was extremely easy and sensitive using low-light video-image analysis. The expression of the firefly luciferase gene, driven by the strong cytomegalovirus promoter, could usually be detected in a matter of minutes, in some cases after only a few seconds. This gene cassette, in concert with the highly sensitive detection system provides a very useful marker system for screening large numbers of transformants in a nondestructive way.

Apart from its use as a transformation marker, the luciferase system promises high potential for spatial imaging of promoter activities *in vivo*. Model organisms such as the zebrafish or medaka are particularly suitable for light-emitting reporter enzymes because of their transparency. However, due to the mosaicism in the transformed founder fish, stable lines of transgenic fish must be established before unambiguous analyses of gene regulation can be performed.

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REFERENCES

Alestrom, P., Kisen, G., Klungland, H. and O. Andersen. 1992. Fish gonadtrophin-releasing hormone gene and molecular approaches for control of sexual maturation: development of a transgenic fish model. Mol. Mar. Biol. Biotechnol. 1, 376–379.

Blin, N. and D. W. Stafford. 1976. A general method for isolation of high molecular weight DNA from eukaryotes. Nucleic Acids Res. 3, 2303–2307.

Concordet, J. P. and P. Ingham. 1994. Catch of the decade. Nature 369, 19–20.

Lin, S., Yang, S. and N. Hopkins. 1994. LacZ expression in gremlin transgenic zebrafish can be detected in living embryos. Dev. Biol. 161: 77–83.

Muller, F., Lete, Z., Varadi, L., Menczel, L. and L. Orban. 1993. Efficient transient expression system based on square pulse electroporation and in vivo luciferase assay of fertilized fish eggs. FEBS 324: 27–32.

Mullins, M. C. and C. Nusslein-Volhard. 1993. Mutational approaches to studying embryonic pattern formation in the zebrafish. FEBS 3: 648–54.

Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98: 503–517.

Stuart, G. W., McMurray, J. V. and M. Westerfield. 1988. Replication, integration, and stable germ-line transmission of foreign sequences injected into early zebrafish embryos. Development 103: 403–412.

Tamiya, E., Sugiyama, T., Masaki, K., Hirose, A., Okoshi, T. and I. Karube. 1990. Spatial imaging of luciferase gene expression in transgenic fish. Nucleic Acids Res. 18: 1072.

Westerfield, M., Wegner, J., Jegalian, B., Derobertis, E. M. and A. W. Puschel. 1992. Specific activation of mammalian Hox promoters in mosaic transgenic zebrafish. Genes Dev. 6: 591–598.

Yamamoto, T. 1961. Physiology of fertilization in fish eggs. Int. Rev. Cytol. 12: 361–495.

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NATURAL REARING TECHNOLOGIES FOR INCREASING POSTRELEASE SURVIVAL OF HATCHERY-REARED SALMON

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ABSTRACT

There is growing concern that poor quality hatchery salmonids die at higher rates than their wild counterparts. We hypothesized that natural rearing environments (raceways fitted with overhead cover, instream structure, substrate, and nonintrusive feed delivery systems) would improve postrelease survival of hatchery salmonids by decreasing rearing stress, reducing domestication, and better acclimating fish to their postrelease environment. We developed and evaluated the effectiveness of a natural rearing system (NATURES) using three postrelease survival experiments conducted on chinook salmon (*Oncorhynchus tshawytscha*). In the first experiment, fall chinook salmon were reared for 4 months, from swim-up to smoltification, in 400-L raceways with cover, structure, and substrate. Postrelease survival to a collection weir 2.2 km downstream was 51% higher for these fish than for conventionally-reared controls. In a second experiment, spring chinook salmon were reared in NATURES-modified and conventional 400-L raceways in clear water for the 3 months preceding smoltification. Postrelease survival to a collection weir 225 km downstream was 24% higher for these fish than for their conventionally-reared cohorts. However, there was no significant difference in postrelease survival between test and control groups reared in turbid water. In the final experiment, culture vessel size was increased to 5,947-L, and fall chinook salmon were reared for about 3 months (from swim-up to smoltification) in raceways outfitted with cover, structure, substrate, and an underwater feed-delivery system. These fish averaged 26% higher postrelease survival to a collection weir 21 km downstream than their conventionally-reared counterparts. We concluded that the more natural, cryptic, body-camouflage coloration observed in NATURES fish contributed to their higher postrelease survival in the stream environment by reducing predator vulnerability. NATURES research provides a foundation for the development of conservation hatcheries to protect native fish that must be taken into culture to rebuild their natural populations through supplementation releases.

INTRODUCTION

Over 200 wild stocks of Pacific salmon have recently been classified at risk of extinction by the American Fisheries Society, and U.S. fisheries managers have become increasingly aware of the need to preserve and rebuild these stocks (Nehlson et al. 1991). In many cases, ecosystem preservation alone will not be adequate to rebuild wild stocks quickly enough to prevent extinction. Theoretically, the fastest way to increase population numbers is through supplementation releases of hatchery-propagated fish. Unfortunately, techniques for producing hatchery-reared fish suitable for rebuilding wild runs are poorly understood.

Most attempts at supplementation that have used hatchery-reared fish to rebuild naturally spawning populations of Pacific salmon have yielded poor results (Moring 1986, Miller 1990, Cuenco et al. 1993). Although the protective hatchery environment does increase egg-to-smolt survival, postrelease survival of cultured salmonids is often considerably lower than that of wild-reared fish (Greene 1952, Miller 1952, Salo and Bayliff 1958, Reimers 1963, Chilcote et al. 1986, Nickelson et al. 1986). Conventional hatchery practices may induce domestication and anomalous behavioral characteristics, which are often considered prime factors in reducing the fitness of hatchery fish for survival in natural ecosystems (Reisenbichler and

McIntyre 1977, Nickelson et al. 1986, Hillman and Mullan 1989, Goodman 1990, Waples 1991, Hilborn 1992).

Present hatchery practices are geared toward mass production under unnatural conditions: fish are reared in the open, over uniform concrete substrate; no structure is provided with which to seek refuge from water current, predators, or dominant conspecifics; fish are held at high, stress-producing densities and are conditioned by surface feeding to approach large, moving objects at the surface (Leitritz and Lewis 1980, Piper et al. 1982). Physiological, behavioral, and morphological modifications resulting from this unnatural rearing environment are probably major factors in the poor postrelease survival of standard hatchery-reared salmon.

Culture strategies for increasing postrelease survival by reducing the vulnerability of hatchery fish to predators were recently reviewed by Maynard et al. (1995). They cited Donnelly and Whoriskey (1991) who showed that brook trout (*Salvelineus fontinalis*) were less vulnerable to predators when challenged over background colors similar to those over which they were reared. In addition, studies by Thompson (1966) and Olla and Davis (1989) have suggested that exposure to predators during culture decreased predator vulnerability after release.

Here we describe research with chinook salmon (*Oncorhynchus tshawytscha*) to develop hatchery rearing procedures that allow cultured fish to maintain their wild characteristics by decreasing rearing stress, reducing domestication, and better acclimating fish to their postrelease environment (Maynard et al. in prep.). The premise of our research is that culture of Pacific salmon in raceways fitted with overhead cover, instream structure, substrate, and nonintrusive feed delivery systems will produce fish with physiological, behavioral, morphological, and survival characteristics similar to those of their wild cohorts. We have conducted three independent studies comparing the postrelease survival of chinook salmon reared in conventional hatchery-type raceways to that of fish reared in raceways outfitted with our natural rearing system (NATURES).

STUDY DESCRIPTION

1992 FALL CHINOOK SALMON STUDY

The first study was conducted in 1991. Fall chinook salmon were reared in 12 rectangular, 400-L acrylic aquaria, set up as laboratory-scale raceways at a National Marine Fisheries Service (NMFS) facility on Big Beef Creek (BBC) near Seabeck in western Washington State. The outside of both ends, the rear, and the bottom of aquaria for control fish were covered with sheets of grey-black, painted polystyrene, to simulate the grey, concrete background coloration of conventional raceways. Each aquarium was supplied with 4 L/min of flowing, 10°C well water.

Aquaria for NATURES fish were similarly outfitted, but more stream-like environments were created by adding 1) plastic aquarium plants and live watercress root wads for instream structure, 2) opaque covers to simulate overhanging banks, and 3) either sand or pea-gravel natural substrates. In this study, there were four replicates for the conventional treatment and eight NATURES replicates. Four of the NATURES replicates were reared over sand and four over pea-gravel, for a total of three treatments (Table 1).

Each tank was stocked with 40 fall chinook salmon swim-up fry, which were reared for 4 months, from swim-up to smoltification as 0-age fish in Spring 1992. Fish in all treatments were fed a standard, prepared pellet diet, presented on the surface by hand. Survival during culture averaged nearly 100% for fish in both conventionally-reared and NATURES treatments. At the end of the culture period, all study fish were anesthetized, measured, and weighed. There was no statistically significant difference ($P>0.05$) in length or weight between conventionally-reared and NATURES fish (Table 1).

On the same day we euthanized and photographed every third fish from each treatment for cryptic coloration analysis. Image analysis indicated that the base integument coloration of fish from all three treatments had a similar brightness component (barren $=21.572$, gravel $=21.730$, sand $=21.710$) ($P=0.942$), but the chroma (barren $=0.377$, gravel $=0.396$, sand $=0.392$) and hue (barren $=0.351$, gravel $=0.364$, sand $=0.362$) of NATURES salmon was significantly ($P=0.000$) different from that of conventionally-reared fish. The grey-scale rank of parr marks was similar for fish from all three treatments. Subjective observations, made over the last 2 months of rearing, indicated that fish reared in NATURES treatment tanks consistently displayed a more olive-brown coloration, larger and darker parr marks, and darker spots than fish reared in conventional treatment tanks. Fish in the conventional treatment tanks always appeared light tan and had poorly developed parr marks and fewer noticeable spots.

On average, the parr marks of NATURES fish reared over gravel occupied a significantly greater percentage of body surface than the parr marks of NATURES fish reared over sand and conventionally reared fish ($P=0.018$). This percentage appeared to increase with increasing coarseness of the grain over which fish were reared: fish reared over course-grained gravel had the largest parr marks (6.3%), and those reared over fine-grained sand had the next largest parr marks (5.8%);

Table 1. Rearing and postrelease survival information for conventionally reared and NATURES fish, 1992–1994

Study	Number of replicates	Size of fish at release		Number of fish released	River length to recapture (km)	Recovery at weir (%)	Relative recovery proportion (Δ%)
		length (mm)	weight (g)				
1992	NATURES = 8	74.6	4.2	203	2.2	60.1*	+51.0
fall chinook	conventional = 4	74.5	4.4	88	2.2	39.8	
1994	*Release 1*						
spring chinook	NATURES = 6	131.0	23.6	437	225	27.2	+24.2
	conventional = 6	133.0*	24.9*	443	225	21.9	
	Release 2						
	NATURES = 6	131.0	23.1	448	225	30.6	−10.1
	conventional = 6	134.0*	24.5*	451	225	33.7	
1994	*Release 1*						
fall chinook	NATURES = 1	69.7	nd	455	21	54.6*	+19.0
	conventional = 1	70.4	nd	423	21	45.9	
	Release 2						
	NATURES = 1	76.1	nd	392	21	44.5	+13.2
	conventional = 1	76.0	nd	467	21	39.3	
	Release 3						
	NATURES = 1	80.3	nd	396	21	45.8*	+66.5
	conventional = 1	81.3	nd	454	21	27.5	

* Indicates statistically significant difference between treatments ($P < 0.05$). For the 1992 fall chinook study, size was analyzed by ANOVA and recovery differences were analyzed by 2 × 2 contingency analysis. For the 1994 spring and fall chinook studies, size was analyzed by Student's t-test and recovery differences were analyzed by 2 × 2 contingency analysis.

however, these parr marks were not substantially larger than those of fish reared over extremely fine-grained conventional tank bottoms (5.6%).

About 65% of the fish were tagged with passive integrated transponder (PIT) tags (Prentice et al. 1990) and subsequently released into Anderson Creek near Seabeck, Washington. These smolts were challenged to survive a 2.2-km outmigration to an estuarine weir. Anderson Creek is a small coastal stream with a heavily wooded riparian zone. The main piscivorous predators observed in the vicinity of Anderson Creek were great blue herons (*Aredea herodias*), kingfishers (*Ceryle alcyon*), mergansers (*Lophodytes cucullatus* and *Mergus* spp.), garter snakes (*Thamnophis spp*), sculpins (*Cottus* spp.), cutthroat trout (*O. clarki*), and rainbow trout (*O. mykiss*).

Outmigration was monitored for more than 84 days, and thereafter, most of the creek was electrofished to ensure that study fish had not taken up residence within the creek. Compared to recoveries of conventionally-reared fish, a significantly greater proportion of NATURES fall chinook salmon were recovered at the weir (60.1 vs. 39.8%) ($P = 0.007$) (Table 1). This equated to a 51% higher relative survival of NATURES fish than conventionally-reared fish. Most fish were recovered at the weir within 3 days of release into Anderson Creek. As there were no weir failures and no chinook salmon were captured by electrofishing, recovery presumably represented survival.

We concluded that predation caused most postrelease mortality, as 1) outmigration was rapid, 2) no chinook salmon appeared to take up residence within the creek, and 3) no fish were found dead or moribund at the weir. The distinctive heterogeneous coloration of NATURES fish should have enhanced their crypticity for the stream bed of Anderson Creek, and thus survival should have been higher for NATURES fish than for their homogeneously colored counterparts. We believe that this enhanced crypsis contributed substantially to the higher recovery/survival rates observed for NATURES fish.

1994 SPRING CHINOOK STUDY

The second study was in 1994. In this experiment, spring chinook salmon were exposed to NATURES rearing at BBC for only a few months prior to smolting. Study fish were reared in 400-L acrylic aquaria, as previously described. However, in this study NATURES aquaria were provided with ornamental junipers (*Juniperous horizontalis*) for instream-structure, pea-gravel over undergravel filters for substrate, and black opaque aquarium covers for overhead cover. There were 2 treatment groups, with 12 replicates for the conventional treatment and 12 NATURES replicates (Table 1).

Each aquarium was stocked with 80 Age-0 spring chinook salmon that had been reared for the previous 9 months in conventional culture. Fish were reared in

study aquaria for 3 months, until smolting as age-1 fish in Spring 1994. Fish in all treatments were fed a standard prepared pellet diet, presented at the surface by hand. The in-culture survival of fish from both treatments exceeded 99%.

There was no significant difference in size of fish between treatments at the beginning of the experiment. However, at the end of the culture period, conventionally reared fish were slightly, but significantly longer and heavier than NATURES fish ($P=0.000$) (Table 1). According to our subjective observations, fish reared in the NATURES aquaria displayed more olive-brown coloration, larger and darker parr marks, and darker spots than fish reared in the conventional treatment tanks.

Approximately 92% of fish were PIT tagged, released in the upper Yakima River, and challenged to survive a 225-km outmigration to a fish collection facility at Prosser Dam on the lower Yakima River. The Yakima River is the third largest river system in Washington State, carrying in excess of 3.70×10^9 m^3 of runoff annually. This river flows through diverse habitats of eastern Washington ranging from subalpine to high mountain desert.

The NATURES and conventional treatment fish were released in two paired groups, with six replicates from each treatment. The first paired release occurred under clear-water conditions, with an estimated Secchi disk reading of over 3 m. The second release occurred under turbid-water conditions, with an estimated Secchi disk reading of less than 15 cm. Most tagged fish that survived to Prosser Dam were detected at the facility within 2 weeks of their release into the upper Yakima River.

From the clear-water release, a higher proportion of the NATURES-reared than conventionally-reared fish were recovered at Prosser Dam (27.2 vs. 21.9%) (Table 1). This equated to an observed 24% higher relative survival for NATURES fish than for conventionally-reared fish. Although not quite statistically significant at the 5% level ($P=0.072$), this finding is encouraging, as it is similar to our 1992 study observations and lends further support to the hypothesis that NATURES fish are more cryptic than conventionally-reared fish against a heterogenous riverine background in clear water.

In the second release, made under turbid water conditions that created a homogenous riverine background, there was a slightly higher, but clearly nonsignificant recovery of fish at Prosser Dam from the conventional treatments than from the NATURES treatments (33.7% vs. 30.6%; $P=0.285$). This 10% higher relative survival for conventionally-reared fish is probably due to chance. However, it may also be indicative that the brighter uniformly colored conventionally-reared fish tended to blend into a light homogenous background better than the darker heterogenous-colored NATURES fish. Therefore, we conservatively recommend that further research clarifying this turbid water finding be conducted prior to adoption of NATURES rearing protocols where fish will be regularly released into turbid water.

1994 FALL CHINOOK STUDY

The third study was conducted in conjunction with the Washington Department of Fish and Wildlife (WDFW) in 1994. In this study, fall chinook salmon were reared in six rectangular, 5,947-L, fiberglass portable raceways at the WDFW Simpson Salmon Hatchery in the Chehalis River Basin of western Washington State. These concrete grey-colored raceways were 6.4 m long by 1.5 m wide by 0.6 m deep. Each raceway was supplied with river water flowing at the rate of approximately 140 L/min.

The three conventional treatment raceways represented a conventional culture environment lacking any substrate, structure, or overhead cover. However, they were covered with translucent bird netting on a plastic pipe frame to prevent bird entry. Conventional-treatment fish were fed a standard pellet diet at the surface by hand.

In the three NATURES raceways, overhead cover was provided by securing military camouflage netting to a plastic pipe frame; this covered the outer 40% of the area on each side of the raceway. The remaining 20% area in the center of the raceways was covered with translucent bird netting to prevent predator entry. This cover configuration simulated the canopy produced by riparian vegetation along streams. The bottom of each NATURES raceway was covered with a 10-cm layer of pea gravel over undergravel filters, which were constructed from a perforated aluminum plate on a 5-cm-high aluminum box frame. Instream structure was created by placing five heavily branched, small (1.0–1.5 m) sheared Douglas fir (*Pseudotsuga menziesii*) trees in each NATURES raceway. Needles were removed from all trees before they were added to the raceways. An automatic rotary-arm hopper feeder dispersed a standard pellet diet into water flowing through a 2.5-cm diameter pipe, which encircled the perimeter of the raceway. The feed-delivery pipe laid over the substrate and delivered food through 7-mm diameter holes drilled at 0.5-m intervals along the topside of the pipe.

Each raceway was stocked with 6,000 fall chinook salmon swim-up fry. Fish were reared in these raceways for about 3 months, until smolting as age-0 fish in Spring 1994. Survival during culture averaged over 98% for fish in both the conventional and NATURES treatments. When ponded, fish from the two treatment groups did not significantly differ in size. However, by

day 59, conventional fish were almost significantly longer (63.7 mm vs 61.9 mm) (P=0.051) and significantly heavier (2.7 g vs 2.4 g; P = 0.007) than NATURES-reared fish.

After observing this difference, we discovered that the subsurface feeders in NATURES raceways had failed to deliver an estimated 10% of the pellet ration. We estimated that the growth advantage for conventional fish approximately matched what the subsurface feeder failed to deliver and concluded that if fish from the NATURES treatment had been presented with an equivalent food ration, their growth would have been similar.

To alleviate this situation as a possible source of bias, conventional fish were not fed for several days to allow NATURES fish to attain equal size. At release, NATURES fish were similar in length and weight to conventionally-reared fish (Table 1).

As in our previous studies, the body color of NATURES-reared fish was noticeably more vivid than that of conventionally-reared fish. According to our visual observations, coloration of fish within treatments was similar, regardless of raceway, while cryptic coloration of fish between treatments contrasted strongly. The integument color of NATURES fish visually matched the brown substrate they were reared over, while that of conventional fish visually matched the light grey of the raceway bottom. The NATURES fish had more extensive melanophore development in the caudal fin, anal fin, abdominal area, and gill cover margin than conventionally reared fish. In addition, the parr marks of NATURES fish were more pronounced than those of conventionally-reared fish.

Beginning in June 1994, fish were released in three paired groups with one replicate from each treatment in each group. Releases were made at 1-week intervals into Bingham Creek, and fish were challenged to survive outmigration to a WDFW weir approximately 21 km downstream. Bingham Creek is a large coastal stream with both logged and unlogged riparian habitat. Juvenile salmonid predators observed in the stream during the study period included river otter (*Lutra canadensis*), great blue heron, belted kingfishers, and steelhead.

The first paired release was made on 13 June 1994, during a period of rainfall. The second release was made on 20 June 1994, during a period with no rainfall and stable creek conditions. The third paired release was made on 27 June 1994, with a period of rainfall following several days later. On each release day, fish from a pair of test and control raceways were crowded, netted, and loaded into an insulated 2,000-L tank with oxygenated water for transport. Each release was liberated into Bingham Creek just before dusk (at approximately 2200 h). In the first few days after release, personnel examining fish trapped at the weir felt they could distinguish treatment fish by body coloration, but these color differences appeared to diminish within a few days after release Conventionally-reared fish apparently initiated development of cryptic coloration similar to that of NATURES fish.

The chinook salmon smolt outmigration was monitored at the trap for 88 days, and the trap was designed to sample 100% of downstream migrants. In the first release, a significantly higher proportion of the NATURES fish than conventionally-reared fish were recovered at the Bingham Creek weir (54.6 vs. 45.9%; P = 0.012) (Table 1). This equated to 19% higher relative survival of NATURES fish over conventionally-reared fish. In the second release, a higher but non-significant, proportion of the NATURES fish than conventionally-reared fish were recovered at the weir (44.5 vs. 39.3%; P=0.138) (Table 1). This equated to a 13% higher relative survival rate for NATURES-reared fish than for conventionally-reared fish. In the third release, a significantly higher proportion of the NATURES-reared fish than conventionally-reared fish were recovered at the weir (45.8 vs. 27.5%) (P<0.001) (Table 1). This equated to more than a 66% higher relative survival for NATURES fish than for conventionally-reared fish.

Overall, a significantly greater proportion of NATURES fish than conventionally-reared fish were recovered at the weir (48 vs. 38%, P<0.001). This equated to an overall relative survival rate that was 26% higher for NATURES fish than for conventionally-reared fish for this study.

The difference in daily recovery of fish at the weir observed between treatments was greatest immediately after release and diminished with time. As noted above, conventionally-reared fish often did not begin to develop camouflage coloration for the stream environment until several days to weeks after release. Experiments have established that the background color pattern of the environment affects both short- and long-term camouflage coloration of salmonids. Short-term physiological color changes are accomplished by chromatophore expansion: pigment is dispersed within the chromatophore unit, and color change occurs within minutes. In contrast, morphological color changes take weeks to complete, since pigments and chromatophore units must be developed to match the general background coloration (Fuji 1993). Therefore, fish that have not developed the morphological color changes required to blend in with the stream background prior to release are the most visible targets after release. Theoretically, those conventionally-reared fish that sought cover and did not migrate until they had developed proper cryptic coloration for their new environment were less vulnerable to visual predators.

DISCUSSION

In nature, stream-dwelling Pacific salmon (e.g., chinook salmon) are solitary and prefer habitats that include small-particle substrates, instream structure as well as overhead cover in the form of aquatic vegetation, fallen trees or undercut tree roots, and undercut banks (Lister and Genoe 1970, Everest and Chapman 1972, Brusven et al. 1986, Hillman et al. 1987). Nevertheless, conventional salmonid hatchery practices are geared toward mass-production of fish under unnatural conditions (e.g., fish are reared in the open, over uniform concrete substrate; no structures are provided with which to seek refuge from water current, predators, or dominant conspecifics; fish are held at high, stress-producing densities, surface fed, and conditioned to approach large moving objects at the surface) (Leitritz and Lewis 1980, Piper et al. 1982).

These practices are thought to give rise to long-term domestication (Reisenbichler and McIntyre 1977, Nickelson et al. 1986). Moreover, conventional hatchery rearing practices can also cause primary behavioral and morphological modifications in cultured fish that may increase predator vulnerability (Thompson 1966, Olla and Davis 1989, Donnelly and Whoriskey 1991). Healey (1991) indicated that predation is a major source of mortality for chinook salmon, and it appears that avian predators are the greatest threat to newly released hatchery fish (Elson 1962, Wood 1987). Maynard once observed that a single heron fed on over 80 similar sized trout within a few hours. However, losses to predatory fish and reptiles may also be significant.

The three main antipredator strategies available to an animal are 1) to avoid areas where predators are found, 2) to escape predators when attacked, and 3) to use cryptic coloration to avoid detection by predators. There is no reason to believe fish from any rearing treatment in our studies would have been better able to avoid areas where predators were found, since all fish had access to the same stream environments after release. In addition, the similarity in size of fish from each treatment suggests that their ability to flee from predators should have been about equal. Thus, our preliminary conclusion is that the primary survival advantages noted for NATURES-reared fish in our studies (Table 1) were related to crypsis.

In all three studies the NATURES-reared fish developed light and dark mottled body camouflage coloration patterns that were cryptic for the diverse stream-bottom background over which these fish were released. Conventionally-reared fish developed a uniformly light coloration that enabled them to blend in with the light grey background coloration of their rearing tank. We believe conventionally-reared fish were cryptically mismatched for their release environment, and potentially more vulnerable to visual-hunting predators (e.g., birds and fish). In previous NATURES studies, Flagg observed that salmonids reared with overhead cover displayed greater fright responses to overhead movement than conventionally-reared groups. Similar enhanced-fright responses were noted for NATURES-reared fish in the 1994 fall chinook study, and these responses may have contributed to their increased survival.

Even though we have not yet completely determined the causal factor(s) increasing postrelease survival of NATURES-reared fish, our studies indicate that NATURES rearing can, at times, confer a valuable instream survival advantage over conventional fish-rearing techniques, at least during clear-water river conditions favorable to visual-hunting predators. The high pre-release survival of both conventional and NATURES fish in all three studies suggests that the NATURES culture techniques we tested did not adversely affect fish health (Table 1).

NATURES components are designed to be retrofitted to existing hatchery raceway systems (Maynard et al. 1995), and vacuuming substrates is the only NATURES raceway operation procedure requiring increased maintenance effort. NATURES research provides a foundation for the development of conservation hatchery concepts for protection of native fish when they must be taken into culture to rebuild natural populations through supplementation releases.

Development of NATURES protocols appears to offer the best hope for producing hatchery fish that are physiologically, behaviorally, and morphologically equivalent to their wild-reared counterparts. Our research indicated that the postrelease survival of cultured fish can be increased by rearing them in an environment that promotes full development of the body camouflage patterns fish will need to avoid predation after release.

LITERATURE CITED

Brusven, M. A., W. R. Meehan, and J. F. Ward. 1986. Summer use of simulated undercut banks by juvenile chinook salmon in an artificial Idaho channel. N. Am. J. Fish. Manage. 6: 32–37.

Chilcote, M. W., Leider, S. A., and J. J. Loch. 1986. Differential reproductive success of hatchery and wild summer-run steelhead under natural conditions. Trans. Am. Fish. Soc. 115: 726–735.

Cuenco, M. L., T. W. H. Backman, and P. R. Mundy. 1993. The use of supplementation to aid in natural stock restoration. *In* J. C. Cloud and G. H. Thorgaard (editors), Genetic Conservation of Salmonid Fishes, p. 269–293. Plenum Press, New York.

Donnelly, W. A. and F. G. Whoriskey, Jr. 1991. Background-color acclimation of brook trout for crypsis reduces risk of predation by hooded mergansers *Lophodytes cucullatus*. N. Am. J. Fish. Manage. 11: 206–211.

Elson, P. F. 1962. Predator-prey relationships between fish-eating birds and Atlantic salmon. Bull. Fish. Res. Board Can. 133.

Everest, F. H. and D. W. Chapman. 1972. Habitat selection and spatial interaction by juvenile chinook salmon and steelhead trout in two Idaho streams. J. Fish. Res. Board Can. 29: 91–100.

Fuji, R. 1993. Coloration and chromatophores. *In* D. H. Evans (editor), The Physiology of Fishes, p. 535–562. Academic Press, New York.

Goodman, M. L. 1990. Preserving the genetic diversity of salmonid stocks: a call for federal regulation of hatchery programs. Environ. Law 20: 111–166.

Greene, C. W. 1952. Results from stocking brook trout of wild and hatchery strains at Stillwater Pond. Trans. Am. Fish. Soc. 81: 43–52.

Healey, M. C. 1991. Life history of chinook (*Oncorhynchus tshawytscha*). *In* C. Groot and L. Margolis (editors), Pacific Salmon Life Histories, p. 311–394. Univ. British Columbia Press, Vancouver, B.C., Canada.

Hilborn, R. 1992. Hatcheries and the future of salmon in the Northwest. Fisheries 17: 5–8.

Hillman, T. and J. Mullan. 1989. Effect of hatchery releases on the abundance and behavior of wild juvenile salmonids. *In* Don Chapman, Inc., Summer and Winter Ecology of Juvenile Chinook Salmon and Steelhead Trout in the Wenatchee River, Washington, 20 p. (Available from Chelan County Public Utility District, Wenatchee, Washington 98801 USA.)

Hillman, T. W., J. S. Griffith, and W. S. Platts. 1987. Summer and winter habitat selection by juvenile chinook salmon in a highly sedimented Idaho stream. Trans. Am. Fish. Soc. 116: 185–195.

Leitritz, E. and R. C. Lewis. 1980. Trout and salmon culture (hatchery methods). Calif. Dep. Fish Game Fish Bull. 164, 197 p.

Lister, D. B., and H. S. Genoe. 1970. Stream habitat utilization by cohabiting underyearlings of chinook (*Oncorhynchus tshawytscha*) and coho (*O. kisutch*) salmon in the Big Qualicum River, British Columbia. J. Fish. Res. Board. Can. 27: 1215–1224.

Maynard, D. J., T. A. Flagg, and C. V. W. Mahnken (editors). In prep. Development of a natural rearing system to improve supplementation fish quality, 1991–1994. Report to Bonneville Power Administration, Contract DE-AI79-91BP20651. (Available from National Marine Fisheries Service, Coastal Zone and Estuarine Studies Division, Box 130, Manchester, Washington 98353 USA.)

Maynard, D. J., T. A. Flagg, and C. V. W. Mahnken. 1995. A review of seminatural culture strategies for enhancing the postrelease survival of anadromous salmonids. Am. Fish. Soc. Symp. 15: 307–314.

Miller, R. B. 1952. Survival of hatchery-reared cutthroat trout in an Alberta stream. Trans. Am. Fish. Soc. 81: 35–42.

Miller, W. H. (editor). 1990. Analysis of salmon and steelhead supplementation. Report to Bonneville Power Administration, Contract DE-A179-88BP92663. 119 p. (Available from Bonneville Power Administration, Box 3621, Portland, Oregon USA 97208.)

Moring, J. R. 1986. Stocking anadromous species to restore or enhance fisheries. *In* R. H. Stroud (editor), Fish Culture in Fisheries Management, p. 59–74. Proceedings of A Symposium on the Role of Fish Culture in Fisheries Management. Amer. Fish. Soc. Bethesda, Maryland USA. 479 p.

Nehlson, W., J. E. Williams, and J. A. Lichatowich. 1991. Pacific salmon at the crossroads: stocks at risk from California, Oregon, Idaho, and Washington. Fisheries 16(2): 4–21.

Nickelson, T. E., M. F. Solazzi, and S. L. Johnson. 1986. Use of hatchery coho salmon (*Oncorhynchus kisutch*) presmolts to rebuild wild populations in Oregon coastal streams. Can. J. Fish. Aquat. Sci. 43: 2443–2449.

Olla, B. L., and M. W. Davis. 1989. The role of learning and stress in predator avoidance of hatchery-reared coho salmon (*Oncorhynchus kisutch*) juveniles. Aquaculture 76: 209–214.

Piper, R. G., I. B. McElwain, L. E. Orme, J. P. McCraren, L. G. Fowler, and J. R. Leonard. 1982. Fish hatchery management. 517 p. (Available from U.S. Fish and Wildlife Service, Washington, D.C.)

Prentice, E. P., T. A. Flagg, and C. S. McCutcheon. 1990. Feasibility of using implantable passive integrated transponder (PIT) tags in salmonids. Am. Fish. Soc. Symp. 7: 317–322.

Reimers, N. 1963. Body condition, water temperature, and overwinter survival of hatchery reared trout in Convict Creek, California. Trans. Am. Fish. Soc. 92: 39–46.

Reisenbichler, R. R. and J. D. McIntyre. 1977. Genetic differences in growth and survival of juvenile hatchery and wild steelhead trout, *Salmo gairdneri*. J. Fish. Res. Board Can. 34: 123–128.

Salo, E. O. and W. H. Bayliff. 1958. Artificial and natural production of silver salmon, *Oncorhynchus kisutch*, at Minter Creek, Washington. Wash. Dep. Fish. Res. Bull. 4.

Thompson, R. 1966. Effects of predator avoidance conditioning on the postrelease survival rate of artificially propagated salmon. Ph.D. Thesis, Univ. Washington, Seattle. 155 p.

Waples, R. S. 1991. Genetic interactions between wild and hatchery salmonids: lessons from the Pacific Northwest. Can. J. Fish. Aquat. Sci. 48 (Suppl. 1): 124–133.

Wood, C. C. 1987. Predation of juvenile Pacific salmon by common merganser (*Mergus merganser*) in eastern Vancouver Island. II: predation of stream-resident juvenile salmon by merganser broods. Can. J. Fish. Aquat. Sci. 44: 950–959.

Bull. Natl. Res. Inst. Aquacult., Suppl. 2: 79-83 (1996)

MARKING EMBRYONIC AND NEWLY EMERGED SALMONIDS BY THERMAL EVENTS AND RAPID IMMERSION IN ALKALINE-EARTH SALTS

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ABSTRACT

One of the enduring fish marking challenges faced by salmon biologists has been how to rapidly and benignly mark large numbers of embryonic or newly emerged fry. In this paper we describe two techniques developed in our laboratory that successfully mark juvenile salmonids. One, thermal marking of otoliths, can be used in incubation facilities to mass mark salmonids from the eyed-stage of development through yolk absorption. The other, immersion in solutions of alkali and alkaline-earth salts, can mark fish at the moment of fertilization and from hatching thereafter.

Thermal marks are induced in the microstructure of otoliths by exposing developing salmon to controlled and abrupt shifts in incubation water temperature. Each time an abrupt temperature decline occurs, an optically dense band is produced in the microstructure of the otoliths. Initially we induced simple, regular patterns into the otoliths of marked fish. Later, when demands for multiple marks arose, we used bar code symbologies to systematically create thousands of recognizable patterns. Thermal marking, particularly the production of codes possessing multiple bands and spaces, requires control over the thermal incubation regime for a month or more. Even with this constraint, we have marked over a 100 million cultured salmon fry using this method. These marks are readily detected in the otoliths of adult salmon; however, fish must be sacrificed in order to collect their otoliths.

In wild populations, and in some other instances, it is impossible to control the temperature regimes experienced by developing fry. In North America, for instance, controlled-flow streams or spawning channels annually produce millions of salmon fry. Evaluating the productivity of these facilities has been very difficult because suitable fry marking methods have not been available. Earlier investigators demonstrated that one-month or longer immersion periods in dilute concentrations (e.g. 1 ppm) of strontium chloride created recognizable deposits of strontium in the scales and otoliths of young salmon. We modified this approach and immersed salmon fry for 12 h or less in baths containing up to 9000 ppm strontium chloride. Fish held for as little as 30 min in 9000 ppm strontium chloride solutions had recognizable deposits of strontium in their otoliths. These marks appear to be detectable throughout the fish's lifetime as individuals held for two years had visible marks. The value of any marking technique increases if multiple marks can be produced. Our analyses indicate that by varying exposure times and marking bath concentrations it is possible to produce strontium bands that have discrete quantities of this element. Similar studies were conducted using chlorides of rubidium, manganese, and a suite of lanthanide elements. This work showed that rubidium was also rapidly incorporated into the calcified tissues of juvenile salmon. In combination, thermal and elemental marking allow investigators to produce cryptic permanent marks on cultured as well as naturally produced salmon fry without apparent stress.

INTRODUCTION

Successful management of salmonid fisheries requires, among other things, the capacity to recognize specific groups of fish. Such recognition helps determine distribution patterns and fishery contribution rates. Marked individuals can also be used to quantitatively evaluate the success of fish cultural and harvest management strategies. These generic needs have prompted the development of many ingenious methods of fish marking and tagging (Wydoski and Emery 1983; McFarlane et al. 1990). Most, however, are applicable only to relatively large individuals ($\geq$ 90 mm) and usually require that the fish be individually handled. In some instances it is desirable to permanently mark embryonic salmonids or newly emerged fry. These

small fish ($\leq$ 42 mm) are easily stressed and large quantities must be marked because of the high natural mortality they typically experience. These two attributes have made small salmonids particularly difficult to mark.

Because these fishes have a protracted developmental period, several months are available for applying marks to cultured fish prior to emergence. This opportunity is generally not available for fry produced under natural conditions. Instead, they must be rapidly marked (within hrs) to minimize behavioral and physiological impacts. Here, we describe two techniques we have used to mark embryonic and newly emerged salmon fry. One induces marks into the microstructure of otoliths by using planned abrupt changes in incubation temperatures from the "eyed-stage" of development through yolk absorption and is thus suitable for cultured fish. The other relies on the rapid deposition of chemicals into the calcified tissues of salmon and was developed to accommodate the needs of marking wild or freely migrating salmonids. Both produce cryptic marks that are retained throughout life and are based on the amplification or purposeful manipulation of natural physiological processes. The objectives of this paper are to briefly review these methods and their applications.

MARKING THE OTOLITHS OF PRE-EMERGENT SALMON BY THERMAL EVENTS

Otoliths (earstones) are the first calcified tissues to appear in salmonid embryos (Neilson et al. 1985; Campana and Neilson 1985) developing in fluid-filled sacs that are part of the inner ear labyrinth. Salmonids have three pairs of otoliths (sagitta, lapillus, and asteriscus) which function in spatial orientation and sound detection (Lowenstein 1971; Popper and Platt 1993). In early ontogeny, small kernels of calcium carbonate and organic material form in each sac. By the "eyed-stage" of development, these have coalesced into a single primordial core to which layers of material are regularly added (Neilson et al. 1985). Pannella (1971; 1974) and subsequently numerous others (for reviews see Gjosaeter et al. 1984; Campana and Neilson 1985; Jones 1985) found that many teleosts deposit otolith increments on a daily basis. Each increment consists of two parts, an incremental zone that is predominately made up of calcium carbonate and a discontinuous zone that contains a fibrous organic matrix of the protein otolin (Campana and Neilson 1985; Maisey 1987). When viewed with transmitted light, the incremental zone is a relatively broad translucent band whereas the discontinuous zone is a typically narrow black or brown opaque band (Campana and Neilson 1985).

Many studies have been conducted to discover the factors that influence the appearance and periodicity of otolith increments. These investigations revealed that photoperiod regimes (Taubert and Coble 1977; Radtke and Dean 1982), temperature (Brothers 1981; 1985; Neilson and Geen 1985), feeding cycles (Neilson and Geen 1985; Volk et al. 1984), and metabolic rates (Mosegaard and Titus 1987; Mosegaard et al. 1988; Wright 1991; Wright et al. 1991) may affect their periodicity or appearance. During early ontogeny, developing salmon feed endogenously and are deleteriously impacted by light (e.g. see Smith 1916; Bell and Hoar 1950; Eisler 1957; Brannon 1965). Water temperature, on the other hand, can be easily manipulated and, if kept at 1° to 13°C from the eyed-stage of development through emergence, will not adversely affect developing salmon.

Fortuitously, temperature changes induce recognizable features in the otoliths of embryonic salmonids. Neilson and Geen (1985) observed that more regular and easily distinguished increments were produced in sectioned sagittae of pre-emergent chinook salmon when the fish were exposed to a variable temperature cycle. Under such a regime, the discontinuous zones in the increments were wider than those produced in fish exposed to a constant water temperature environment. Brothers (1985) also discovered that abrupt drops in temperature produced optically dense increments in the sagittae of larval lake trout.

In 1985 we began a series of experiments to determine whether an artificially produced water temperature cycle could be used to induce recognizable marks on the otoliths of incubating salmon. These experiments (Volk et al. 1990; Volk et al. 1994) showed that an optically dense increment was rapidly induced in the otoliths of embryos and alevins after they had been exposed to an abrupt drop in water temperature. Inspection of sectioned otoliths from adult salmon marked by this method demonstrated that these bands were retained throughout a fish's life. Otolith marking by this method may commence anytime after the individual primordial elements have fused into a single primordial core, usually when a visible eye is present beneath the chorion of the developing salmon egg.

One of the most important factors influencing the ultimate clarity of a thermally induced otolith mark is the ambient temperature regime a fish experiences while it is being marked. When distinctive diurnal temperature fluctuations occur highly contrasted otolith increments are produced and it becomes difficult to distinguish bands induced by artificial temperature changes. Under these circumstances, we typically expose fish to non ambient conditions for 24 to 72 h. Conversely, a diurnally stable water temperature regime

results in weakly contrasted increments against which artificially induced marks are easily identifiable. In this case, temperature changes of 2°C for a matter of hours will produce recognizable marks.

In general, we strive to make the actual otolith mark consist of a number of individual thermal events. There are a variety of ways to create such patterns and we originally constructed patterns as sets of evenly spaced thermal events. However, as the demand for unique otolith codes increased, we began looking for a set of systematic rules that could be used to create unambiguous thermal marks. In Volk et al. (1994) we described how a bar code symbology, the Interleaved Two of Five Rule (ITF), could be employed to create as many as 1000 otolith banding patterns. In using this rule six thermal events are used to create six optically dense otolith increments and five intervening spaces. Two of these spaces are designed to be at least twice as wide as the other three, directly proportional to the time between thermal events. Altogether, 10 possible combinations of narrow and wide spaces can be created. In a typical hatchery-produced salmonid otolith, there is sufficient time to induce three sets of these six band patterns, making 1000 codes possible. By adding or subtracting one or two thermal events or by changing the number of wide and narrow spaces present in a six band unit, many additional codes also become possible. Care should be taken to ensure that hatching does not occur during the establishment of a six band pattern, for an optically dense band that closely resembles a thermal mark is produced at hatching (Volk et al. 1990).

The utility of a mark also depends on how easily it can be decoded. Using the methods briefly described below, it is possible for one person to decode several hundred otoliths a day. After an otolith (usually sagitta in juveniles and sagitta or lapillus in adults) has been dissected, it is laid medial surface down on a glass plate, then embedded into a polyester block. If all the otoliths placed on a plate are from similarly-sized fish (fork lengths that are within 5 mm of one another), 10 or more can be ground and polished simultaneously. If they originated from adult fish or from individuals with disparate body lengths, they should be sectioned individually. A diamond saw is used to cut the plastic blocks into smaller pieces when individual sectioning is necessary. To decode a specimen, the polyester blocks are held by hand (otolith-side down) over a spinning lap wheel supplied with 500-grit silicon carbide paper. The specimens are ground until the primordial core is visible under a low power dissecting microscope. The specimen is then polished using polishing cloth and 1-μm aluminum oxide slurry on a spinning lap wheel. Polished hemi-sections are read using transmitted light at 100 to 400 X. Thermal marks can be decoded by computerized pattern recognition algorithms or by human eye (Volk et al. 1994). We commonly re-read 100% of our specimens to ensure that accurate decodes have been made. When conflicts occur, both readers re-examine the specimen and make a final determination. Where poor preparations result in no decodes, the opposite otolith may be retrieved for processing.

Typically we thermally mark over 30 million juvenile salmon a year. These fish are being used to address a wide array of fishery management, research, and ecological questions. Our methods are also being used by Canadian and Alaskan researchers. Hagen and Munk (1994) recently reported that over 300 million thermally marked salmon have been released into Alaskan waters.

MARKING SALMON FRY AND FINGERLINGS BY IMMERSION IN ELEMENTAL SALTS

In some instances, such as in natural spawning areas and controlled-flow spawning channels, thermal marking may not be feasible due to the difficulties of precisely manipulating the temperature of such large water volumes. Yet to evaluate survival, growth, migration patterns, and other parameters, millions of individuals must be rapidly marked. In the past, various fin clips (Bergstedt 1985) and half-length (0.5 × 0.25 mm) binary-coded wire have been used to mark and tag salmon fry (Thrower and Smoker 1984). These methods are labor intensive and often not practical for newly emerged salmon because of their small size (20–42 mm FL).

A reasonable mass-marking alternative appears to be the passive introduction of alkaline and rare earth elements into the calcified tissues of salmon fry. Trefethen and Novotny (1963) suggested that selected stable isotopes be used to mark juvenile salmon either by immersion or by feeding fish diets laden with bone-seeking cations. Since their recommendation, investigators have fed (e.g. Ophel and Judd 1968; Behrens Yamada et al. 1979; Michibata 1981; Behrens Yamada and Mulligan 1982; Guillou and de la Noüe 1987; Muncy et al. 1988) and injected (e.g. Michibata and Hori 1981; Brothers 1990) elements into fishes or immersed them (e.g. Muncy and D'Silva 1981; Behrens Yamada and Mulligan 1987; Brothers 1990; Snyder et al. 1992; Ennevor and Beames 1993) into solutions containing dissolved elemental salts, all of which successfully incorporated higher levels of these elements into the calcified tissues of treated fish.

Of the three delivery systems used, direct immersion appears to be the best method to rapidly mark small salmon. Most previous studies (yet see Brothers 1990) that have marked fish by immersion, used solutions with low concentrations of an element and prolonged periods

of exposure. Behrens Yamada and Mulligan (1987), for instance, held coho fry for 49 days in waters containing 1 ppm of strontium chloride ($SrCl_2$). This treatment produced a ten-fold increase of strontium (Sr) in the fishs' vertebrae.

In 1990, we modified the marking procedures of Behrens Yamada and Mulligan (1987) by immersing newly emerged sockeye and chum salmon fry into concentrated (120, 1200, and 9000 ppm) solutions of $SrCl_2$ for 24 h (Schroder et al. 1995). We hypothesized that Sr would be rapidly absorbed by the fry and then deposited in their calcified tissues. After being immersed, the fry were reared in freshwater for 5 wk. Vertebrae, opercula, and otoliths (sagittae) were collected from treated and control fish and analyzed with Inductively Coupled Plasma Mass Spectrometry (ICPMS). Control fish had about 200 ppm of Sr in their calcified tissues while chum salmon fry exposed to the two most concentrated Sr baths had approximately 1600 ppm in their vertebrae and opercula and over 4000 ppm of Sr in their otoliths. Even the most dilute immersion treatment (120 ppm) successfully marked the calcified tissues of chum and sockeye fry. We attempted to facilitate the marking process by using hyperosmotic baths, DMSO, and heat. In some cases these adjuvants did increase the uptake of strontium. However, in many instances, fry immersed in Sr solutions without them retained as much or more strontium than treated counterparts (Schroder et al. 1995).

When strontium-exposed otolith sections were viewed with back scattered electron microscopy, a highly visible band of deposited strontium was observed. Electron beam micro-analysis (Wave Dispersive Spectrometry) disclosed that peak strontium counts in the bands equalled 110,000 ppm in chum fry immersed in 9000 and 1200 ppm solutions while those held in 120 ppm baths had peak counts that ranged from 48,000 to 68,000 ppm (Schroder et al. 1995). An additional group of sockeye fry were immersed into a 5000 ppm solution of $SrCl_2$ and reared for 21 months in fresh water. Back scattered electron images taken of otolith sections collected from these fish also showed clear strontium marks (Schroder et al. 1995) demonstrating that strontium marks produced at the fry stage were easily detected for at least 2 y.

Since this work was completed, we have conducted a number of additional elemental marking experiments (S.L. Schroder, C.M. Knudsen, and E.C. Volk unpublished data). The results of these studies have shown that strontium marks can be produced if fry are immersed for just 1 h in a 9000 ppm solution of $SrCl_2$. We also immersed fall chinook fingerlings into concentrated solutions of Rubidium, Manganese, and various rare earth (Cerium, Gadolinium, Lanthanum, and Samarium) chlorides for 24 h. The scales, vertebrae, and otoliths of fish exposed to a 90 ppm bath of rubidium chloride were all marked with this element. Exposure to a 45 ppm bath of Manganese chloride also left recognizable deposits of manganese in otoliths and vertebrae but not in the scales of treated fish. The rare earth chlorides we tried were generally toxic when concentrations exceeded 10 ppm and in only one case did we see any apparent uptake of one of these elements (Samarium) into calcified tissues after a single 24 h exposure period. Our interest in these elements was prompted by the work of Ennevor and Beames (1993) and Ennevor (pers. comm.) who showed that solutions containing very low concentrations (50 to 100 ppb) of rare earth acetates could be used to mark salmonid juveniles if the fish were subjected to a multiple week exposure period.

Finally, a review by Alderdice (1988) suggested that salmonid fishes incorporate some cations (e.g. Na, Ca, and K) into their eggs at fertilization and for several hours thereafter. To determine whether such a proclivity could be used to mark salmon, we activated eggs and sperm in 5000 ppm solutions of $SrCl_2$ and held the fertilized gametes in this solution for an additional 3 h. ICPM analyses of otoliths taken from these fish indicated that the individuals held in our strontium solutions had significantly higher levels of this element than control fish. Consequently, simple immersion into various elemental salts at fertilization and shortly thereafter may be another method that can be used to mark embryonic salmonids.

These studies and others that are currently being performed in our lab have shown that direct immersion in alkaline earth solutions for relatively short periods of time ($\leq$ 1 h) can permanently mark newly emerged Pacific salmon fry. In combination, thermal and elemental marking are finally providing researchers with several nonstressful marking tools that can be used on both cultured (thermal and elemental) and naturally produced (elemental) salmon embryos and fry.

LITERATURE CITED

Alderdice, D. F. 1988. Osmotic and ionic regulation in teleost eggs and larvae. *In* W. S. Hoar, and D. J. Randall (eds.), Fish physiology, vol. XI, part A pp. 163–251. Academic Press, San Diego.

Behrens Yamada, S., and T. J. Mulligan. 1982. Strontium marking of hatchery reared coho salmon *Oncorhynchus kisutch* Walbaum, identification of adults. J. Fish Biol. 20: 5–9.

Behrens Yamada, S., and T. J. Mulligan. 1987. Marking nonfeeding salmonid fry with dissolved strontium. Can. J. Fish. Aquat. Sci. 44: 1502–1506.

Behrens Yamada, S., T. J. Mulligan, and S.J. Fairchild. 1979. Strontium marking of hatchery-reared coho salmon (*Oncorhynchus kisutch*, Walbaum). J. Fish Biol. 14: 267–275.

Bell, G. M., and W. S. Hoar. 1950. Some effects of ultra violet radiation on sockeye salmon eggs and alevins. Can. J. Res. Sect. D. Zool. Sci. 28: 35–43.

Bergstedt, R. 1985. Mortality of fish marked by fin clipping; an annotated bibliography, 1934–1981. U. S. Fish Wildl. Serv., Great Lakes Fish. Lab., Adm. Rep. No. 85–3: 40 pp.

Brannon, E. L. 1965. The influence of physical factors on the development and weight of sockeye salmon embryos and alevins. Int. Pac. Salmon Fish. Comm. Prog. Rep. 12. 26 pp.

Brothers, E.B. 1981. What can otolith microstructure tell us about daily and sub-daily events in the early life history of fish? Rapp. P.-V. Réun., Cons. Int. Explor. Mer 178: 393–394.

Brothers, E. B. 1985. Otolith marking techniques for the early life history stages of lake trout. Great Lakes Fishery Commission, Research Completion Report, Ann Arbor, MT.

Brothers, E. B. 1990. Otolith Marking. Am. Fish. Soc. Stet. 7: 183–202.

Campana, S. E. and J. D. Neilson. 1985. Microstructure of fish otoliths. Can. J. Fish. Aquat. Sci. 42: 1014–1032.

Ennevor, B. C., and R. M. Beames. 1993. Use of lanthanide elements to mass mark juvenile salmonids. Can. J. Fish. Aquat. Sci. 50: 1039–1044.

Eisler, R. 1957. Some effects of artificial light on salmon eggs and larvae. Trans. Am. Fish. Soc. 87: 151–162.

Guillou, A., and J. de la Noüe. 1987. Use of strontium as a nutritional marker for farm-reared brook trout. Prog. Fish-Cult. 49: 34–39.

Gjosaeter, J., P. Dayaratne, O. A. Bergstad, H. Gjosaeter, M. I. Sousa, and I. M. Beck. 1984. Ageing tropical fish by growth rings in the otoliths. FAO Fish. Circ. No. 776. FAO, Rome.

Hagen, P. and K. Munk. 1994. Stock separation by thermally induced otolith microstructure marks, p. 149–156. In Proceedings of the 16th Northeast Pacific Pink and Chum Salmon Workshop. Alaska Sea Grant Coll. Prog. Rep. No. 94–02.

Jones, C. 1985. Determining age of larval fish with the otolith increment technique. Fish. Bull. 84: 91–98.

Lowenstein, O. 1971. The labyrinth. In W.S. Hoar and D.J. Randall (eds.), Fish physiology, vol. 5, pp. 207–240. Academic Press, New York, NY.

Maisey, J. G. 1987. Notes on the structure and phylogeny of vertebrate otoliths. Copeia 1987: 495–499.

McFarlane, G. A., R. S. Wydoski, and B. E. Wright. 1990. Historical review of the development of external tags and marks. Am. Fish. Soc. Symp. 7: 9–29.

Michibata, H. 1981. Labeling fish with an activable element through their diet. Can. J. Fish. Aquat. Sci. 38: 1281–1282.

Michibata, H., and R. Hori. 1981. Labeling fish with an activable element. Can. J. Fish. Aquat. Sci. 38: 133–136.

Mosegaard, H., and R. Titus. 1987. Daily growth rates of otoliths in yolk sac fry of two salmonids at five different temperatures. *In* S.O. Kullander and B. Fernholm (eds.), Proc. Fifth Conf. Eur. Ichtyol. Stockholm, 1985 pp. 221–227. Swedish Museum of Natural History, Stockholm.

Mosegaard, H., H. Svedäng, and K. Taberman. 1988. Uncoupling of somatic and otolith growth rates in arctic char (*Salvelinus alpinus*) as an effect of differences in temperature response. Can. J. Fish. Aquat. Sci. 45: 1514–1524.

Muncy, R. J., and A. P. D'Silva. 1981. Marking walleye eggs and fry. Trans. Am. Fish. Soc. 110: 300–305.

Muncy, R. J., N. C. Parker, and H. A. Poston. 1988. Marking striped bass with rare earth elements. Proc. Annu. Conf. Stet. Assoc. Fish Wildl. Agencies 41: 244–250.

Neilson, J. D., and G. H. Geen. 1985. Effects of feeding regimes and diel temperature cycles on otolith increment formation in juvenile chinook salmon, *Oncorhynchus tshawytscha*. Fish. Bull. 83: 91–101.

Neilson, J. D., G. H. Geen, and D. Bottom. 1985. Estuarine growth of juvenile chinook salmon (*Oncorhynchus tshawytscha*) as inferred from otolith microstructure. Can. J. Fish. Aquat. Sci. 42: 899–908.

Ophel, I. L., and J. M. Judd. 1968. Marking fish with stable strontium. J. Fish. Res. Board Can. 25: 1333–1337.

Pannella, G. 1971. Fish otoliths: daily growth layers and periodical patterns. Science 173: 1124–1127.

Pannella, G. 1974. Otolith growth patterns: an aid in age determination in temperate and tropical fishes. *In*: T.B. Bagenal (ed.), Proc. Inter. Symp. Ageing of Fish, pp. 28–39. Unwin Brothers Ltd. Surrey.

Popper, A. N. and C. Platt. 1993. Inner ear and lateral line of fish. In: D.H. Evans, (ed), The Physiology of Fishes. pp. 99–136. CRC Press, Boca Raton, FL.

Radtke, R. L., and J. M. Dean. 1982. Increment formation in the embryos, larvae and juveniles of the mummichog, *Fundulus heteroclitus*. Fish. Bull. 80: 41–55.

Schroder, S. L., C. M. Knudsen, and E. C. Volk. 1995. Marking salmon fry with strontium chloride solutions. Can J. Fish. Aquat Sci. 95: 1141–1149.

Smith, E.V. 1916. Effect of light on the development of young salmon. Puget Sound Mar. Sta. Publ. 1: 89–107.

Snyder, R. J., B. A. McKeown, K. Colbow, and R. Brown. 1992. Use of dissolved strontium in scale marking of juvenile salmonids: effects of concentration and exposure time. Can. J. Fish. Aquat. Sci. 49: 780–782.

Taubert, B. D., and D. W. Coble. 1977. Daily rings in otoliths of three species of *Lepomis* and *Tilapia mossambica*. J. Fish. Res. Bd. Canada 34: 332–340.

Thrower, F. P., and W. W. Smoker. 1984. First adult return of pink salmon tagged as emergents with binary-coded wires. Trans. Am. Fish. Soc. 113: 803–804.

Trefethen, P. S., and A. J. Novotny. 1963. Marking fingerling salmon with trace-elements and non-radioactive isotopes. Int. Comm. N.W. Atl. Fish. Spec. Publ. 4: 64–65.

Volk, E. C., R. C. Wissmar, C. A. Simenstad and D.M.Eggers. 1984. Relationship between otolith microstructure and the growth of juvenile chum salmon (*Oncorhynchus keta*) under different prey rations. Can. J. Fish. Aquat. Sci. 41 (1): 126–133.

Volk, E. C., S. L. Schroder, and K. L. Fresh. 1990. Inducement of unique otolith banding patterns as a practical means to mass-mark juvenile Pacific salmon. Am. Fish. Soc. Symp. 7: 203–215.

Volk, E. C, S. L. Schroder, J. J. Grimm, and S. Ackley. 1994. Use of a bar code symbology to produce multiple thermally induced otolith marks. Trans. Am. Fish. Soc. 123: 811–816.

Wright, P. J. 1991. The influence of metabolic rate on otolith increment width in Atlantic salmon parr, *Salmo salar* L. J. Fish Biol. 38: 929–933.

Wright, P. J., D. Rowe, and J. E. Thorpe. 1991. Daily growth increments in the otoliths of Atlantic salmon parr, *Salmo salar* L., and the influence of environmental factors on their periodicity. J. Fish Biol. 39: 103–113.

Wydoski, R., and L. Emery. 1983. Tagging and marking. *In* L.A. Nielsen and D.L. Johnson (ed.), Fisheries Techniques, pp. 215–237. American Fisheries Society, Bethesda, MD.

Bull. Natl. Res. Inst. Aquacult., Suppl. 2: 85-87 (1996)

INTERNATIONAL COOPERATION IN WORLD AQUACULTURE

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ABSTRACT

International cooperation has always been the hallmark of productive science. Through joint scientific ventures with colleagues from other nations and through far-reaching research programs into questions of national and international interest, the California Sea Grant College System seeks the benefits of cooperative efforts. This presentation will focus on exemplary examples of international collaboration supported by Sea Grant.

INTRODUCTION

The dynamic growth of the Pacific Rim has resulted in strongly competitive relations. And yet, I would like to suggest that competitive relations do not necessarily imply adversarial relations.

I believe that the potential of the Pacific region will be met when the Eastern and Western cultures that compose it capitalize on each other's strengths. Even now the region is characterized by a remarkable willingness of its member societies to learn from one another. And it is this openness, this internationalism, that will allow the community of the Pacific to develop to its fullest potential.

The program that I direct, the California Sea Grant College System, is part of a national investment in university research and education that works to enhance the U.S. competitive position in the world marketplace in an environmentally friendly manner. But beyond that, by promoting wise use of marine resources, we aim to contribute to the well-being and prosperity of all the world's people. Through joint scientific ventures with colleagues from other nations and through far-reaching research programs into questions of national and international interest, we seek the benefits of cooperative scientific efforts. I have been asked to share with you a few specific examples where California Sea Grant's international cooperation has been very important to the advance of aquaculture and then point to some areas where I see the potential for future collaborative work.

I recognize that I need not remind this audience that taxonomy is the foundation on which aquaculture rests. Whereas the taxonomy of those animal species that are important to aquaculture is with few exceptions well-known, the seaweeds that are of commercial interest are not as well understood. For a decade now, California Sea Grant in collaboration with Sea Grant programs from the other Pacific states, and under the leadership of Dr. Isabella Abbott of the University of Hawaii, has sponsored a series of biennial Pacific Rim workshops focused on the taxonomy of economically important algae of the Pacific. Hosts have included universities in the United States, Japan, the People's Republic of China, and Guam. The next conference will be held in Malaya in July 1995, and if past experience is any guide we can expect working contributions from colleagues located all around the Pacific Rim, including Vietnam and Chile. The progress these scientists are making on such important groups as *Sargassum* and *Gracilaria* is a testimony to the effectiveness of pooling our brain power to attack common problems.

Many of you are aware of the work of Dr. Howard Bern and his colleagues at the University of California, Berkeley. For many years, with Sea Grant support, Dr. Bern studied hormonal control of salmon growth and development. More recently, he has extended this work to striped bass. Because salmon are one of the most valuable fisheries in the northern hemisphere, his research early on attracted collaborators from around the world. Many Japanese collaborators, for example, have spent long periods of time in Dr. Bern's laboratory. In return, Bern and his associates have often visited colleagues in Japan and other parts of the world. This work has been collaborative in the truest sense. To give just one example, Bern's collaborators at the Ocean Research Institute at Tokyo University developed a test

for salmon growth hormone and measured levels of growth hormones for the Berkeley laboratory. His most recent work has been on tilapia, a fish that is increasingly being grown worldwide. Bern is working with collaborators at the Ocean Research Institute, the Hawaii Institute of Marine Biology, UCLA, and Guangzhou University.

Looking to the future, I see a number of possible areas of collaboration on issues related to the culture of salmon and other marine finfish. One example related to ocean ranching of salmon is genetic selection programs to increase rates of return. In the culture of other marine finfish, such as flatfish, we could profit from larval-rearing and early nursery programs to decrease costs prior to ocean-cage culture or even continued land-based culture. In 1990, Drs. Bern, Grau, and Hirano organized an international Sea Grant conference at Scripps Institution of Oceanography on the broad issue of hormonal control of growth in fishes. As a direct outgrowth of this conference, a Sea Grant Working Committee was formed to develop a research agenda in the area of enhancement of fish growth and development. This committee concluded that productivity from traditional capture fisheries and from cultured seafood products can be significantly improved through the development of several specific biotechnologies. Prominent among these is the ability to use naturally occurring hormones and growth factors to maximize growth.

Recent research indicates that endocrine manipulation can safely produce the same kind of remarkable increases in fish and shellfish growth that are now observed in the livestock industry. But capitalizing on this opportunity will require intensive and highly focused fundamental research that will lead to the safe and appropriate use of growth factors. Knowledge of both the main and secondary effects of the endocrine system is also imperative for the rational design of genetically engineered varieties of fish and other seafood. The U.S. Congress has recently recognized the potential of this area as one among several to which the application of biotechnology can make an important contribution with a special appropriation. This is another area where international cooperation could reap substantial benefits. And, to help make this new opportunity a reality, Dr. Chang, Dr. Bern, and Dr. Hirano organized the Sea Grant-sponsored international symposium, "Application of Endocrinology to Pacific Rim Aquaculture" held at Bodega Marine Laboratory, California, in September of 1994. Many Japanese scientists (representing Hokkaido, Nikko, Tokyo, Okazaki, Mie) participated, which pleased us very much and will, we hope, lead to more cooperation. The work of Professor Daniel Morse of the University of California, Santa Barbara, has also involved numerous international collaborators. With Sea Grant support, Morse has investigated the settlement, metamorphosis, and growth of abalone, and his techniques for stimulating abalone spawning and settlement are used in many parts of the world. Morse's collaborators have included scientists from the Oyster Research Institute in Japan, the Institute of Oceanology in the People's Republic of China, and the Central Marine Fisheries Research Institute in India. Most recently, he has been trying to develop a simple method for stimulating the abalone itself to produce growth-accelerating hormones. International collaborators on this aspect of his work represent the Netherlands, Taiwan, Japan, and the People's Republic of China. Morse was also instrumental in our decision to host the Third International Abalone Symposium, which I am pleased to announce will be held in Monterey, California, September 7–13, 1997.

Lastly, I must mention the contributions to international aquaculture of the crustacean specialists we have funded at Bodega Marine Laboratory in California. Dr. Wallis Clark, whose work on the shrimp species *Sicyonia ingentis* is directly transportable to a number of shrimp species cultured worldwide, has made that species a model system for studies on the reproduction of shrimp. Dr. Clark recently moved to the University of Florida, but he is presently involved in a collaborative investigation funded by the National Science Foundation of the United States, the National Science Council of Taiwan, and California Sea Grant. This study seeks to obtain the knowledge by which to enhance hybridization of marine shrimp by overcoming the barriers between eggs and sperm of different shrimp species. Colleagues in this collaboration are Drs. T. -I. Chen and J. -H. Cheng of the Tungkang Marine Laboratory, Dr. Griffin from Bodega Marine Lab, and Dr. Clark. We strongly believe that the domestication of shrimp through their entire life cycle is essential to the future development of this industry and that this continues to be an area where cooperative efforts are badly needed. The collapse of the shrimp industry in a number of regions around the world as a result of environmental degradation and disease also points to the clear need for cooperative work. For example, the dramatic reduction in production and growth of shrimp in Ecuador as a result of a virus calls for the selection and improvement of more resistant animals or the development of virus-free lines that will serve as sound broodstock.

At the UJNR business meeting, Dr. Mahnken suggested some areas for cooperative work on salmon. With his kind permission, I want to suggest these ideas as a framework for cooperation on other species as well. These areas are:

- Techniques for genetic stock identification;
- Culture of captive broodstock;

- Disease and parasite control;
- Ecological and genetic interaction of wild and hatchery fish;
- Climatic and oceanic factors in abundance fluctuation;
- Better understanding of reproduction and physiology and control of reproduction; and
- The impact of aquaculture and enhancement on the environment and water quality.

In closing, I have presented examples of how California Sea Grant has supported, encouraged, and participated in cooperative efforts: sharing our special expertise with the world community of scientists and receiving that community's expertise in turn. Such international cooperation has always been the hallmark of productive science, and we are pleased that UJNR has expressed a strong interest in playing an important role in future international cooperative relationships.

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HIGHLIGHTS OF AQUACULTURE RESEARCH IN THE NATIONAL SEA GRANT PROGRAM

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ABSTRACT

The National Sea Grant College Program supports aquaculture research at 29 coastal and Great Lakes states. The research program is focused on a broad range of aquaculture topics including aquaculture system development, genetics, physiology and endocrinology, nutrition, disease diagnosis and control, and policy and regulations. Marine and Great Lakes species including coldwater, coolwater, and warmwater species are being investigated. Past research has led to the establishment of new industries including hybrid striped bass culture, triploid oysters for the Pacific Northwest, softshell crab culture, hard clam production, improved salmon strains for pen culture, and a variety of other technologies used throughout the aquaculture industry. Several new Sea Grant research developments from different geographic regions of the United States will be discussed. Topics include algal production, effects of beneficial bacteria on water quality and survival, and computer control of aquaculture systems.

OVERVIEW

The National Sea Grant College Program was created by Congress in 1966 to provide for the understanding and wise use of ocean, coastal, and Great Lakes resources and the environment. It consists of a network of over 300 institutions nationwide operating through a core leadership of 29 Sea Grant colleges and institutions. This network annually draws upon the talents of over 3000 scientists, engineers, educators, students, and outreach specialists.

Administratively, the National Sea Grant College Program is headquartered in the U.S. Department of Commerce's National Oceanic and Atmospheric Administration (NOAA). Sea Grant supports coordinated programs of university-based research, education, and technology transfer that support the overall mission of NOAA. In doing this, Sea Grant mobilizes the resources of both the Federal Government and academia in a dynamic partnership to mitigate and solve urgent coastal and ocean problems.

Sea Grant activities in marine ecology and fisheries, aquaculture, coastal processes, environmental studies, seafood science, and the coastal ocean support NOAA's mission. Over the years, Sea Grant has also taken on several missions unique in NOAA such as research and technology transfer in the areas of aquaculture, marine biotechnology, and marine policy.

For 25 years, Sea Grant has taken a strong role in developing aquaculture technology for marine, estuarine, and Great Lakes species. The National Aquaculture Act of 1980 and the National Aquaculture Development Plan of 1983 have provided a basic outline for past Sea Grant research, and Sea Grant researchers are taking an active part in developing a new National Aquaculture Plan for the future. The National Sea Grant Office provides yearly guidelines to Sea Grant institutions in order to focus research proposals on identified priority areas. Sea Grant also supports workshops and symposia for key aquaculture species groups to establish the status of the industry and to focus on research needed to support the developing industry. The overall support of aquaculture-related research has been about $4 million in Federal funds, with another $3 to $4 million in matching funds, and Sea Grant Extension efforts in aquaculture amount to about $1.5 million per year. Approximately 100 individual projects are supported nationwide at this level of effort. The research program is focused on a broad range of aquaculture topics including aquaculture system development, genetics, physiology and endocrinology, nutrition, disease diagnosis and control, and policy and regulations. Marine and Great Lakes species including coldwater, coolwater, and warmwater species are being investigated. Past research has led to the establishment of new industries including hybrid striped bass culture, triploid oysters for the Pacific Northwest, softshell crab culture, hard clam production, improved

salmon strains for pen culture, and a variety of other technologies used throughout the aquaculture industry.

Summarizing the research results of this large program is impossible for this paper, but I will highlight the work done by Dr. Phillip Lee, of the University of Texas Medical Branch, on the automated monitoring and control of aquaculture production systems. This technology is extremely important for the future development of the closed, recirculating aquaculture systems that the U.S. is developing to alleviate the environmental concerns associated with aquaculture.

INTRODUCTION

The modern commercial aquaculture facility has become a sophisticated network of interrelated processes and sub-processes that convert raw materials (oxygen, heat, feed, and water) into a high-quality final product (edible high protein flesh) at a rapid rate. These processes are comparable to the physical processes managed by manufacturing (George 1992). They require many simple (stepwise) and complex (side-loop) processes to be integrated spatially and temporally in order to maximize production and minimize failures (Lee 1993, 1994,1995). As a result, the aquaculture manager and his staff have been compelled to become experts in biochemistry, animal physiology, microbiology, animal behavior, engineering and construction as well as animal caretaking, the last one being their primary job.

The application of process control technology and the concurrent need for aquaculture-specific intelligent management systems are central to continued intensification of the aquaculture industry. Automated intensive aquaculture systems will allow the industry to: (1) locate production sites closer to markets, (2) improve environmental control, (3) reduce catastrophic losses, (4) avoid problems with environmental regulations on effluents, (5) reduce management and labor costs, and (6) improve product quality and consistency (Lee 1995). Another major advantage of automated control systems is in the scale-up from pilot plant to production scale. All poorly understood processes require pilot-scale operations before significant expansion to production, allowing the operators to test different control paradigms (Dowling and Sullivan 1993). The automated control system produces a global, real-time database that speeds control model refinement because the enhanced quality of the data obtained reduces the number of empirical trials needed to perfect the control model (≈40% less time required; Nisenfeld 1989).

CURRENT STATUS

Four basic designs for automated control systems (Dray 1994) are illustrated in Figure 1.

Lee (1991, 1993) adapted an industrial process control system for use in aquaculture (funded by Texas A&M University, Sea Grant R/ME-1). The system

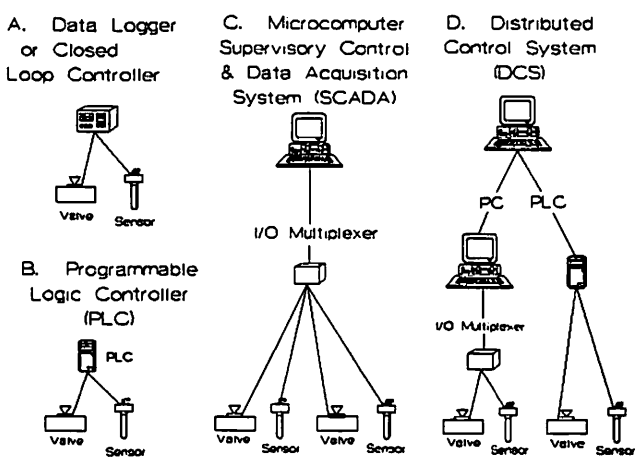

Figure 1. (1A) closed loop controller or data logger systems that are simple, inexpensive, local control systems, lacking communications capabilities but including some data storage (Szabo 1993); (1B) programmable logic controller (PLC) systems that perform control functions at the lowest system level are highly redundant to avoid system failure, do not store the input/output (I/O) as files and have limited display capabilities unless attached to a computer or terminal (Cleaveland 1993); (1C) microcomputer-based supervisory control and data acquisition (SCADA) systems that are dedicated systems allowing real-time analysis (analog and digital) and storage of I/O in a database for historical trending (Bailliet 1987; Yingst 1988); and (1D) distributed control systems (DCS) that provide greater multi-tasking, redundancy, and data storage capacity by networking multiple microcomputers and/or PLCs (George 1992). Closed loop controllers and PLCs (Figure 1A and 1B) are examples of local control systems (control near the process); SCADA (Figure 1C) is a centralized control system; and DCS (Figure 1D) is an integrated control system, combining elements of the other two types (Spennato & Noblett 1992).

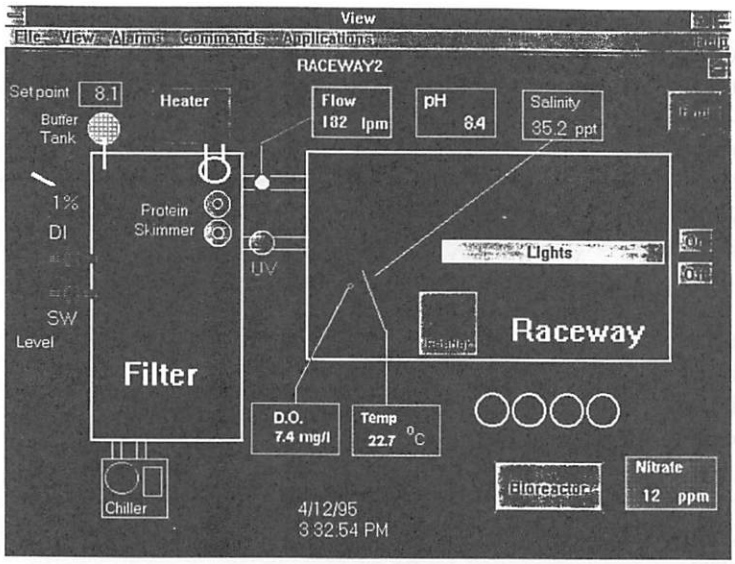

Figure 2. Real-time display of automated raceway system (14,500-L). Addition of buffer, state of lights, addition of sea water (SW) or deionized water (DI) and state of ultraviolet sterilizer (UV) are animated. Bioreactor button links to bioreactor display.

was composed of a microcomputer (SCADA) running commercially available software, an input/output (I/O) multiplexer, and numerous sensors and meters (Figure 2). Temperature, salinity, photoperiod, pH, dissolved oxygen, water flow, and water level were monitored and controlled in a closed, recirculating seawater raceway (Turk and Lee 1991). The system included an automated denitrifying bioreactor for the control of nitrate levels in sea water (Lee 1991, 1993; Whitson et al. 1993). The automated bioreactor is composed of a bacterial bed column, I/O multiplexer with sensor array, carbon feed pump and reservoir, variable-speed main flow pump, and microcomputer (Whitson et al., 1993). The system is controlled using a commercial process control software package and it has been in continuous operation for three years. This system is currently being upgraded to a distributed control system (DCS) that will control multiple aquaculture tank systems in three buildings.

Recent results indicate that automation can be used to realize substantial cost/savings in terms of labor and materials (Lee et al. 1995). The operation of the control system reduces the labor needed to monitor water quality and control system function as well as time required to input collected data and report trends. Estimates were made based on the daily activities of personnel who monitor and operate similar tank systems. These estimates do not include labor for water exchanges or labor for correcting problems associated with catastrophic system's failures. The original installation costs for the SCADA system on a per tank basis (US $5,980) would be offset by savings in labor for operation within two years (US $2,584/yr).

The denitrifying bioreactor operates according to design, and since 1992 the nitrate concentrations in the raceway have never risen above 20 ppm. Water exchange was eliminated and only addition of deionized water caused by evaporation and of seawater caused by losses from cleaning and leaks was required ($\approx$ 0.015%/day). Our prior management practice was to operate the raceway system closed, adding water for evaporation and cleaning losses only, for up to six months and then replace the water completely. The sea water used in the raceway is expensive and annual cost of water replacement has been US $6,000. Operating costs for the bioreactor were estimated to be less than US $1.00 a day (e.g., electricity and methanol) for an annual operating budget of US $365.00. Under these conditions, the bioreactor saves approximately US $5,635 a year. The bioreactor represents approximately 27% of this cost (US $4,900). One must also consider the costs of performing a nitrate test once per week (chemicals and 8 min labor, US $2.20) since this can now be avoided (for 10 tanks, US $1,144/yr). As a result, the bioreactor was able to offset its installation costs within the first year of operation.

THE FUTURE

Several areas of process control technology that are important for aquaculture should experience significant advances during the immediate future. These areas include: improved sensor development, incorporation of artificial intelligence into process control software, and computer or machine vision (Lee 1995). The most significant advances in control technology will come in the methods used to encode control models in software. Software will go far beyond simple on/off control or statistical control, extending into the realm of machine or "artificial" intelligence. The most probable techniques used will be: expert systems (Bechtold 1993, 1994; Eliot 1994) and neural nets (Chester 1992). The

former will require some prior knowledge of the process to be controlled while the latter can learn to control a process on its own. Fuzzy logic control has great possibilities since the types of processes to be controlled in aquaculture are often poorly understood but highly dynamic (Karr 1993). Fuzzy logic control is being used to control operation of the automated denitrifying bioreactor described above (Whitson et al. 1993) with support from a Department of Commerce Small Business Innovation Grant.

Several new types of sensors that have application in environmental monitoring are also on the horizon. Piezoelectric crystal biosensors incorporate an oscillating crystal with a chemical coating sensitive to specific dissolved chemicals so that the analyte concentration adsorbed to the crystal changes the oscillation frequency proportionately (Guilbault and Suleman 1993). Even the more classical amperometric and potentiometric sensors (i.e., pH, ion specific electrodes, redox and conductivity) have seen many improvements as new materials and new designs make it to the market (Gary 1989; Gennett and Purdy 1991). Fiber optic sensors composed of lasers and multiple thin film reagent layers have the advantage of fouling minimization, since antifouling chemicals can be incorporated directly into the reagent layers, so that they constantly leach from the surface (Luo and Walt 1989). Finally, a new generation of smart sensors and actuators are beginning to appear that have integrated circuits incorporating memory, math/logic functions, and communications protocols (Bryzek 1993; Miller and Grumstrup 1993). The remote location of certain aquaculture facilities (i.e., sea cages and large pond systems) also requires the development of more economical, sophisticated communications protocols (i.e., microwave, radio wave or satellite communications protocols).

Machine vision will open up new opportunities to monitor aquatic animal growth and health noninvasively at remote locations. A machine vision subsystem has been added to the above mentioned SCADA control system using a video capture card and an underwater, black-and-white surveillance camera (Whitsell and Lee 1994). The system (funded by Texas A&M University, Sea Grant R/ME-1) has been used to estimate animal activity in real time by using a frame subtraction method followed by immediate classification based on up to 51 features calculated for each shape. The classification technique allowed differentiation of animals from noise, allowing animal movements to be quantified automatically. The vision system is now being used to quantify size and weight of aquatic animals so that instantaneous biomass estimates can be made.

Undoubtedly, the most important objective in designing and installing any modern control system is planning for future compatibility. A control system design should allow for improvements and upgrades as these new technologies become available (Lee 1995).

LITERATURE CITED

Bailliet, R. M. 1987. PC-based SCADA systems spans the Gulf of Mexico. InTech 34 (9): 59–64.

Bechtold, W. R. 1993. A practical guide to expert systems: part a. Instrum. Control Syst. 66(12): 41–43.

Bechtold, W. R. 1994. A practical guide to expert systems: part b. Instrum. Control Syst. 67(2): 75–78.

Bryzek, J. 1993. Smarter, less-costly sensors are on the way. Instrum. Control Syst. 66(10): 85–87.

Chester, D. 1992. New trends in neural nets. Sci. Comput. Autom. 8(6): 43–48.

Cleaveland, P. 1993. PLC users want more. Instrum. Control Syst. 66 (3): 31–45.

Dowling, R. and M. O. Sullivan. 1993. The "hows" and "whys" of PC-based process simulation. Instrum. Control Syst. 66(6): 59–63.

Dray, D. L. 1994. Process control: back to the basics. Instrum-.Control Syst. 67 (1): 67–73.

Eliot, L. 1994. Gravitating toward common sense. AI Expert 9(2): 9–11.

Gary, K. 1989. On-line electrochemical sensors in fermentation. Am. Biotechnol. Lab. 7(2): 26–33.

Gennett, T. and W. C. Purdy. 1991. Electrochemical sensors: part 2, recent advances. Am. Lab. 23(6): 60–66.

George, G. 1992. Distributed control: another way to go. Instrum. Control Syst. 65(10): 35–36.

Guilbault, G. G. and A. Suleman. 1993. Piezoelectric crystal biosensors. Am. Biotechnol. Lab. 8(4): 28–32.

Karr, C. L. 1993. Adaptive process control with fuzzy logic and genetic algorithms. Sci. Comput. Autom. 9(10): 23–30.

Lee, P. G. 1991. Automation of aquaculture systems: a review and practical guide to implementation. In: Proceedings of the Symposium on Engineering Aspects of Intensive Aquaculture, pp. 284–300. Northeast Regional Aquaculture Engineering Service, Ithaca, NY.

Lee, P. G. 1993. Computer automation of recirculating aquaculture systems. In: J. K. Wang (ed.), Techniques for Modern Aquaculture, pp. 61–70. American Society of Agricultural Engineers, St. Joseph, MI.

Lee, P. G. 1994. Computer automation and intelligent control for aquaculture. Bull. Natl. Res. Inst. Aquacult. (Suppl. 1): 105–110.

Lee, P. G. 1995. A review of automated control systems for aquaculture and design criteria for their implementation. Aquacult. Eng. 14: 205–227.

Lee, P. G., P. E. Turk, and J. L. Whitson. 1995. Automated control of a closed, recirculating mariculture system with attached denitrification filter. In: M. B. Timmons (ed.), Aquacultural Engineering and Waste Management, Northeast Regional Aquacultural Engineering Service, Ithaca, NY. NRAES 90: 23–39.

Luo, S. and D. R. Walt. 1989. Fiber-optic sensors based on reagent delivery with controlled-release polymers. Analyt. Chem. 61: 174–177.

Miller, M. D. and B. F. Grumstrup. 1993. Smart valves help firms make smarter decisions. Instrum. Control Syst. 66(1): 59–62.

Nisenfeld, A. E. 1989. Improving pilot plant effectiveness using a distributed control system. Control 11(9): 69–77.

Spennato, N. and F. Noblett. 1992. Integrating PLCs and DCSs via

a computer and relational database. Instrum. Control Syst. 65 (6): 79–82.

Szabo, L. F. 1993. Today's single-loop controllers are packed with big-system features. Instrum. Control Syst. 66 (3): 23–26.

Turk, P. E. and P. G. Lee. 1991. Design and economic analyses of airlift versus electrical pump driven recirculating aquaculture systems. In: M. B. Timmons (ed.), Engineering Aspects of Intensive Aquaculture, Northeast Regional Aquacultural Engineering Service, Ithaca, NY. NRAES 49: 271–283.

Whitsell, A. and P. G. Lee. 1994. A plug-and play machine vision application for aquaculture. Sci. Comput. Autom. 10(8): 29–32.

Whitson, J., P. Turk, and P. G. Lee. 1993. Biological denitrification in a closed recirculating marine culture system. In: J. K. Wang (ed.), Techniques for Modern Aquaculture, pp. 458–466. American Society of Agricultural Engineers, St. Joseph, MI.

Yingst, J. C. 1988. PC-based architecture guide process control. InTech 35(9): 117–120.

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A NEW STRATEGY FOR THE PROPAGATION OF MASU SALMON, *ONCORHYNCHUS MASOU MASOU* (BREVOORT), IN TOYAMA PREFECTURE

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ABSTRACT

Masu salmon, or cherry salmon, *Oncorhynchus masou masou*, is one of the important commercial salmonids in northern Japan, especially on the coast of the Sea of Japan. This salmon is very valuable and expensive especially in Toyama Prefecture. The statistical data on catch of masu salmon in the Jinzu River which is the main river for capturing masu salmon in Toyama Prefecture decreased markedly and rapidly in the past 80 years because of anthroposenic river disturbances.

In order to recover natural stock of masu salmon, a new strategy is planned to utilize the deep sea water of Toyama Bay in rearing masu salmon from smolts to spawning adults with the aim to obtain one million masu salomn eggs to release. By means of heating the deep sea water by underground freshwater through the heat-exchanger systm, we can obtain both sea water and fresh water at optimum water temperature for rearing masu salmon including freshwater and marine periods. In addition, we have planned to utilize river reservations to rear masu salmon from fry to smolts safely and naturally by making temporal streams artificially.

THE ECONOMICAL VALUE OF MASU SALMON AND THE REDUCTIVE TENDENCY OF ITS RESOURCES

Masu salmon, or cherry salmon, *Oncorhynchus masou masou* (Brevoort), is one of the important commercial salmonids in northern Japan, especially on the coast of the Sea of Japan, as well as chum salmon, *O. keta*, and pink salmon, *O. gorbuscha*. In Toyama Prefecture, in central Honshu on the Sea of Japan, there are many rivers where masu salmon used to run such as the Jinzu River, the Shou River, and the Kurobe River (Figure 1). This salmon is very valuable and expensive, and is utilized mainly for "Sushi of masu salmon" which is wrapped in bamboo leaves and pressed (Figure 2). Traditionally a kind of pressed "Sushi" is well-known in this area.

In 1900–1909, the statistics on catch of masu salmon was 160 t in the Jinzu River, but it was only 5 t in 1980s. The catch of masu salmon decreased markedly and rapidly for 80 years (Figure 3). The reduction of masu salmon resources is caused by recent environmental disturbances in rivers, for instance, construction of dams and riverwalls, river improvement, river conservation work, reduction of the amount of flowing water, etc. Masu salmon lost spawning grounds and juvenile growing areas owing to these environmental changes.

THE ECOLOGY AND PROPAGATION OF MASU SALMON

Masu salmon usually spend one year, rarely two years in fresh water area from fry to parr stages before migrating to sea as smolts (Mayama 1992). Smolts appear from the middle of March to late April with the peak in late March at the lower reaches of the Shou River. The fork length distribution of smolts captured at the lower reaches of the Shou River ranges from 12 to 17 cm with a mode of 13 cm (Tago 1993a), and the smoltification rate of juveniles is about 40 to 70% (Tago 1992,1993b).

The main food of juveniles examined at the lower reaches of the Shou River in 1992 consisted of aquatic insects of mayfly nymphs, *Ephemeroptera*, at the rate of 74.4 to 94.3% in wet weights in March, and from early April to mid April, it consisted of chum salmon fry at the rate of 66.6 to 93.7%. In late April, it comprised mayfly nymphs and chum salmon fry at the rate of 43.8% and 40.2%, respectively (Tago 1994).

After one year of marine life, masu salmon return to

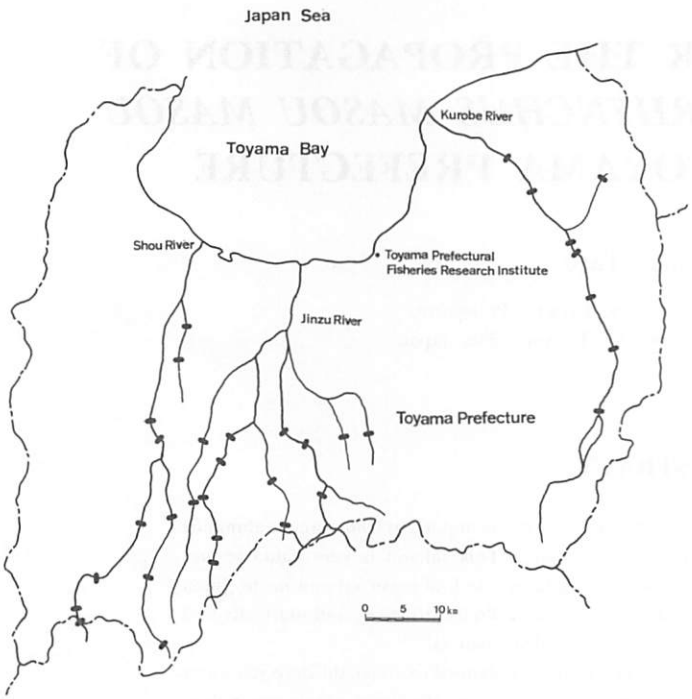

Figure 1. The location of the Jinzu River, the Shou River, the Kurobe River, the Toyama Bay and Toyama Prefectural Fisheries Research Institute. Closed ellipses indicate main dams.

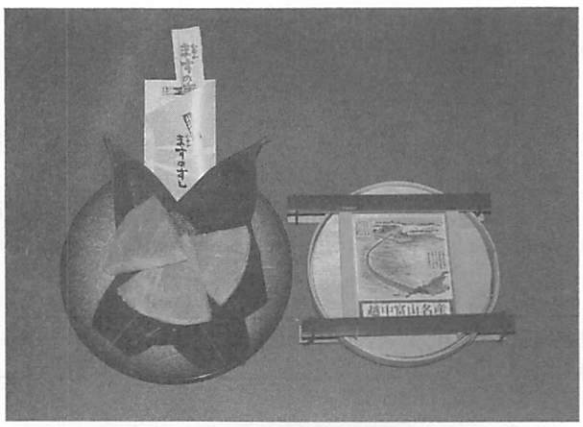

Figure 2. "Sushi of masu salmon".

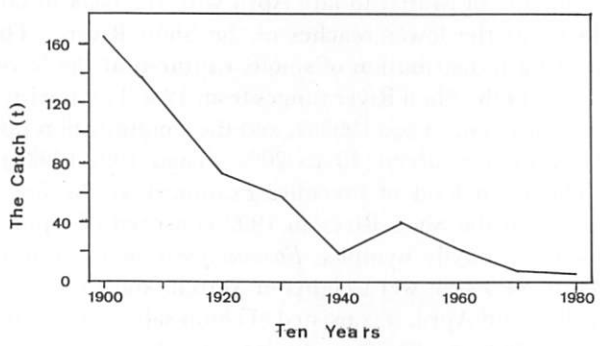

Figure 3. The changes of ten years average catch of masu salmon in the Jinzu River from 1900–1909 to 1980s.

their native river for spawning. The catch of masu salmon starts in Feburary and ends in July with the peak in May. Masu salmon usually hide in deep pools in the river. They are caught by drift nets or casting nets in the river from spring to early summer. The fork length distribution of adults caught in the Jinzu River ranges from 40 to 70 cm with a mode of 60 cm, and the body weight distribution ranges from 1.0 to 4.0 kg with a mode of 3.0 kg. During this time the migrating fish rarely feed. Masu salmon running into the Jinzu River consist of about 70 to 80% females (Tago 1992,1993b). Masu salmon spawn in the upstream areas of the main stem or tributaries from October to November, and end their life for three years.

Artificial rearing of masu salmon fry is conducted at some hatcheries in Toyama Prefecture. At Jinzu hatchery, artificial rearing of masu salmon fry has been conducted since in 1972 (Figure 4), but the catch of masu salmon in the Jinzu River did not increase at all (Figure 3). To restore masu salmon resources, the release of juveniles into the Jinzu River was begun to be promoted by Toyama Prefectural Fisheries Research Institute in 1985 (Figure 4). However, the returning rate and the number of returning fish to native rivers is estimated from 0.1 to 0.3% and only a few hundreds, respectively (Tago 1992,1993b).

To enhance masu salmon resources, there are many subjects to examine such as the shortage of eggs (especially sea run form), the high cost of rearing masu salmon to smolts and the low rate of smoltification (40～70%). The most difficult and urgent issue is the supply of eggs, especially natural native eggs of sea-run masu

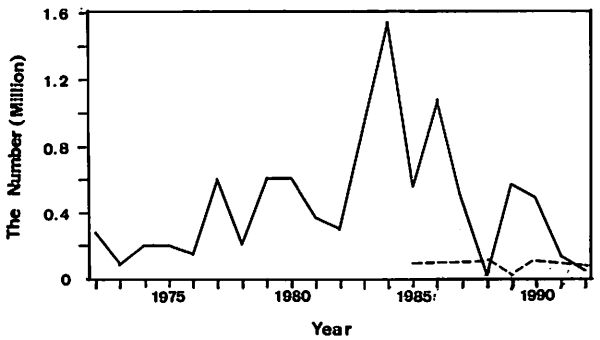

Figure 4. Number of artificially reared masu salmon fry and juveniles released into the Jinzu River from 1972 to 1992. Solid line indicates fry and a broken line indicates juveniles (fingerlings and smolts).

salmon. Many Japanese hatcheries have difficulty in getting sufficient masu salmon eggs to release.

A NEW STRATEGY FOR THE PROPAGATION OF MASU SALMON

We cannot rear masu salmon in sea water throughout a year by the usual method because of high water temperature in the summer. To control it, a new scheme is planned to utilize the deep sea water of Toyama Bay in rearing masu salmon from smolts to spawning adults at our institute.

The properties of pumped-up the deep sea water from 321 m in depth in Toyama Bay are characterized by low water temperature (about 5°C), high concentration of nutrients, poor aerobic bacteria, stability of water quality, etc. The heat-exchanger system can obtain both sea water and fresh water at optimum water temperature for rearing masu salmon (Figure 5). By means of heating the deep sea water (5°C) by underground freshwater (18°C) through this heat-exchanger system, we are able to obtain about 12°C sea water and fresh water respectively. Using temperature controlled sea water and fresh water, we can rear masu salmon thoughout a full life cycle.

The deep sea water can be obtained only 2,630 m off the coast at a depth of 321 m (Figure 6). It costs about one billion yen to construct the facility for fingerling production with the use of 125 t an hour of the deep sea water. This facility with six ponds of 25 t (Figure 7) will be used to rear masu salmon from smolts to spawning adults with the aim to obtain one million eggs of masu salmon. This scheme to use deep sea water in rearing masu salmon may be the first trial in the world.

In addition, we have planned to utilize river reservations (Figure 8) by making a temporal stream to rear masu salmon juveniles as a countermeasure to

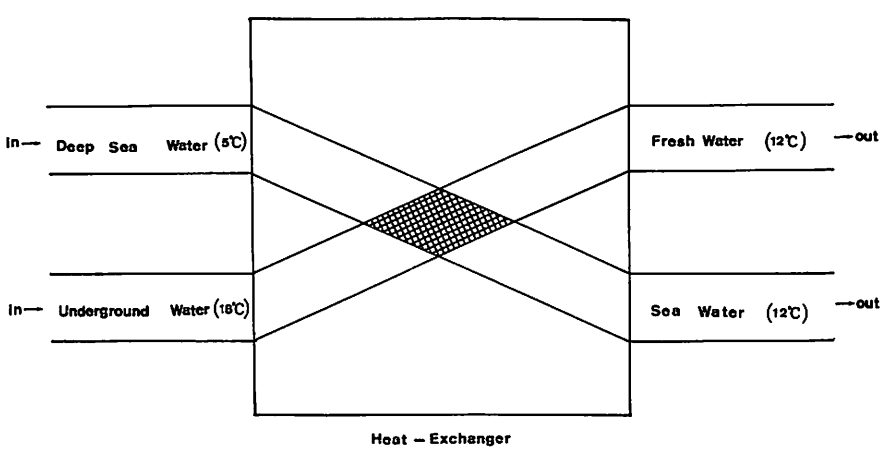

Figure 5. The outline of the heat-exchanger system to get both sea water (12°C) and fresh water (12°C) with deep sea water (5°C) and underground freshwater (18°C) for rearing masu salmon.

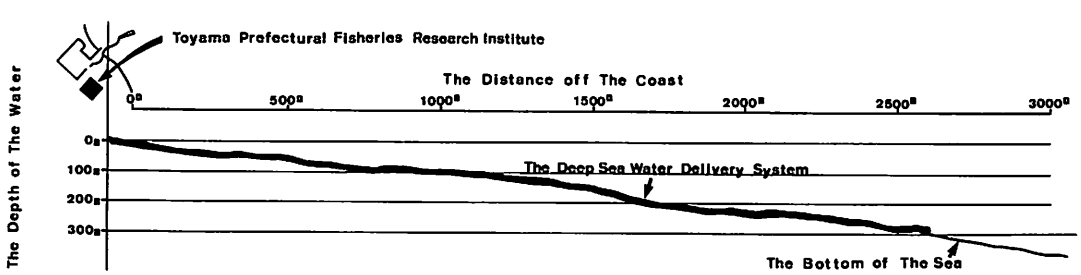

Figure 6. Cross section of deep sea water delivery system (a wide line).

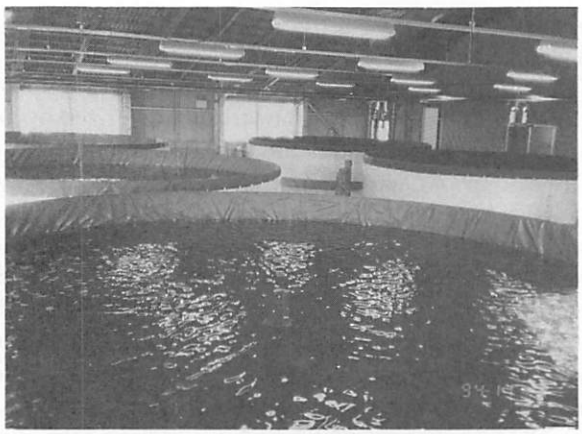

Figure 7. The 25t ponds for rearing masu salmon fingerling production with the use of 125 t an hour of the deep sea water.

Figure 8. A temporal stream inside river reservation in the Kurobe River. The main stream is seen on left side, and a temporal stream is seen on right side.

recent enviromental disturbances in rivers and to the reduction of masu salmon juveniles by casting nets of ayu, *Plecoglossus altivelis*, in summer.

The properties of a temporal stream inside river reservations are full flowing water, stable water temperature, and the low cost of rearing and rearing fish more naturally and safely. In fact, in the Shou River the number of returnning chum salmon adults increased markedly in recent years with the use of temporary streams inside river reservations for rearing chum salmon fry (Tuno 1992).

There are river reservations in many rivers of Toyama Prefecture and we can easily make temporary streams artificially to rear masu salmon from fry to smolts naturally and safely.

By means of supplying eggs obtained from adults reared in the deep sea water to hatcheries and using river reservations for rearing juveniles, we could set about more efficient stock enhancement of masu salmon, and we expect that many rivers in Toyama Prefecture will be fulfilled with masu salmon in the near future.

ACKNOWLEDGMENTS

I thank Dr. Matusato, the director of Toyama Prefectural Fisheries Reserch Institute, for his valuable advice. I wish to express gratitude to Ms. Mayumi Uonomi and Ms. Masako Nakajima for their help in arranging data.

LITERATURE CITED

Mayama H. 1992. Studies on the freshwater life and propagaton technology of masu salmon, *Oncorhynchus masou* (Brevoort). Sci. Rep. Hokkaido Salmon Hatchery No.46 1–156 (In Japanese; English summary).

Tago Y. 1992. Report on masu salmon enhancement survey in Toyama Prefecture in April 1991 – March 1992. Rep. Toyama Pref. Fish. Exp. Stn, 36 pp (In Japanese).

Tago Y. 1993a. The size and the period of seaward migration of masu salmon smolts released into the Shou River, Toyama. Bull. Toyama Pref. Fish. Exp. Stn. No4, 41–52. (In Japanese; English abstract).

Tago Y. 1993b. Report on masu salmon enhancement survey in Toyama Prefecture in April 1992 – March 1993. Rep. Toyama Pref. Fish. Exp. Stn, 41 pp (In Japanese).

Tago Y. 1994. Feeding habit of juvenile masu salmon, Oncorhynchus masou, during the period of seaward migration in the Shou River of Toyama Prefecture. Bull. Toyama Pref. Fish. Exp. Stn. No5, 13–20 (In Japanese; English abstract).

Tuno Y. 1992. Report on chum salmon enhancement survey in Toyama Prefecture in April 1991 – March 1992. Rep. Toyama Pref. Fish. Exp. Stn, 1–18 (In Japanese).

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ON THE RADIUS OF THE FIRST RING ON THE SCALE OF RED SEA BREAM *PAGRUS MAJOR* AS A CHARACTER FOR STOCK SEPARATION IN THE SEA OF JAPAN

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ABSTRACT

A tentative method for separating the red sea bream stocks, *Pagrus major*, is described by means of the radius of the first annual ring on the scales. The radius showed significant differences among the three areas; north stock (off Akita Pref.): 2.66±0.32 mm, central stock (off Kyoto Pref.): 3.80±0.61 mm and south stock (off Nagasaki Pref.): 4.46±0.45 mm. There is a positive correlation (0.912) between the mean radius of the first annual ring and the duration in day for which the individuals were thought to have developed in more than 18°C water temperature (50 m deep). The smallest radius (about 2.7 mm) in the north stock corresponds to the shortest period (less 120 days) while the largest radius (about 4.5 mm) in the south stock to the longest period (210 days). It has been suggested that the differentiation in the radius among three areas are related to water temperatures during the 0 age development stage, namely, the differences in developmental rates of 0 age.

Judging from a series of scale samplings from the sea off Kyoto Pref. (central stock), measurments of the radius and analysis of three stock compositions, it has been implied that the red sea bream migrate to the north area in spring and to the south area in autumn. In the fishing grounds off Kyoto Pref., the north stock of red sea bream (small radius of first ring) appeared with a high percentage occurrence as age increased, about 20% at one year old, about 60% at four years old. The results are expected to prove useful in migration and stock structure studies of the red sea bream, and in age and growth problems.

INTRODUCTION

The red sea bream, *Pagrus major*, is one of the most important commercial species in Japan. In the Sea of Japan artificial seeds of red sea bream had commenced to stabilize the fish catch about 20 years ago. In the sea off Kyoto Prefecture, which is located in the western part of the Sea of Japan, artificial seeds of red sea bream have been released since 1979. Currently, approximately a million artificial seeds of red sea bream are released every year. One of the major problems that has delayed conservation action on the red sea bream stock has been the question of identifying the different spawning stock groups and of the different seeds produced by each local station. Also local stocks possibly mix with each other.

Therefore, we attempted to obtain information on the red sea bream stock separation in the Sea of Japan, by using the first annual ring radius on the scales of fish collected from the northern to the southern fishing grounds.

MATERIALS AND METHODS

A total of 1,698 1-year-old red sea breams were collected from 1992 to 1993 in the areas shown in Figure 1. In addition, a total of 907 2 to 6-year-old red sea breams were collected by set-net, and hand line and long line, from 1992 in the sea off Kyoto Prefecture.

Five to ten scales in each fish were collected from the central part of the left side of the fish, between the upper part of the pectoral fin and lateral line. The radius of the first ring which showed some irregular spacing and patterns was measured along a line from the focus to the basi-lateral angle according to Murakami and Okada (1967). The radius was measured to the nearest one hundredth of a millimeter. At least 5 scales from the same specimen were measured, and the averages were taken as the measured values. The age of individual fish was estimated according to the result of age estimation made by Akazaki (1960).

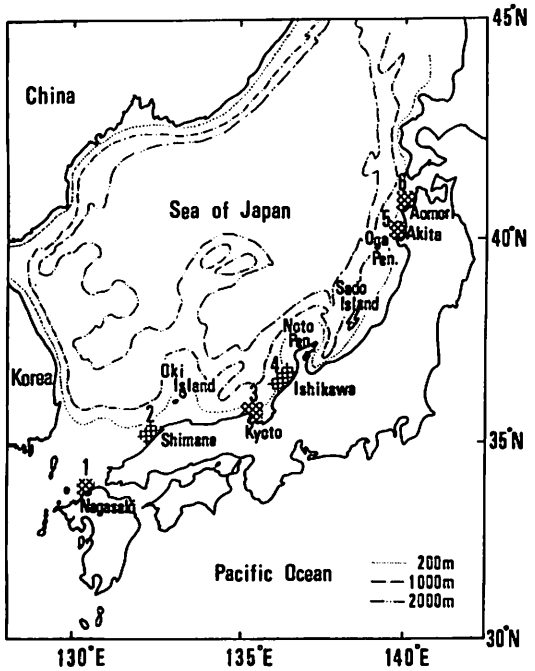

Figure 1. Map of sampling regions of the red sea breams. Number 1–6 shows locations where the fish specimens were collected.

RESULTS

COMPOSITIONS OF THE FIRST RING RADIUS ON THE SCALE

a: 1-year-old fish in each area

For fork length of 1-year-old red sea breams from 6 different fishing grounds, and of measurements of the radius of the first ring on the scales are shown in Table 1. This table shows there is a 470 mm difference in the mean of fork length at 1-year-old fish and a 1.80 mm difference in the mean of radius of the first ring on the scales. The fish with a small radius of the first ring on the scales were collected from the northern sea in Aomori and Akita and those with a large radius from the southern sea in Nagasaki. As a result, the average first ring radius on the scales of 1-year-old fish showed some geographical trends.

The compositions of the first ring radius on the scales of 1-year-old fish in each area are shown in Figure 2. Their compositions in all the areas displayed normal curve, and Mann-Whitney's U testing was then conducted. As a result, no significant differences were observed among 3 compositions of the first ring radius on the scales of 1-year-old fish in the sea off Ishikawa, Akita and Aomori Prefectures. And these 3 compositions have significant differences between the other 3 compositions with 5% significance levels, respectively.

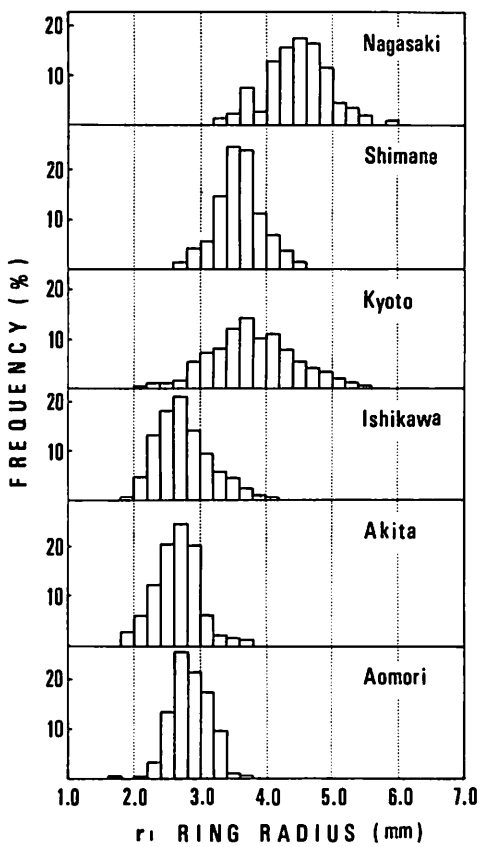

Figure 2. Frequency distribution of the radius of the first ring (r_1) on the scales of the red sea bream in each area.

Table 1. Mean ± standard deviation of fork length (FL) and radius of first ring (r_1) on the scale

	Locality	FL (mm) mean ± S.D.	R_1 (mm) mean ± S.D.
1	Imari Bay of Nagasaki Pref.	164 ± 12.7	4.46 ± 0.450
2	Taisha area of Shimane Pref.	152 ± 9.9	3.60 ± 0.335
3	along the coast of Kyoto Pref.	183 ± 23.7	3.80 ± 0.610
4	Kaga area of Ishikawa Pref.	136 ± 12.7	2.75 ± 0.399
5	Oga kitaura of Akita Pref.	153 ± 6.7	2.66 ± 0.320
6	Otose area of Aomori Pref.	165 ± 10.7	2.83 ± 0.280

The composition of the first ring radius on the scales of 1-year-old fish collected from 6 areas could be grouped into 3 compositions shown in Figure 3. The composition was separated into the 3 normal curves in which the average radius and standard deviations are 4.44 ± 0.33 mm (Group A), 3.66 ± 0.31 mm (Group B), and 2.68 ± 0.28 mm (Group C) by means of the Cassie method (Cassie 1954) and the Taylor method (Taylor 1965).

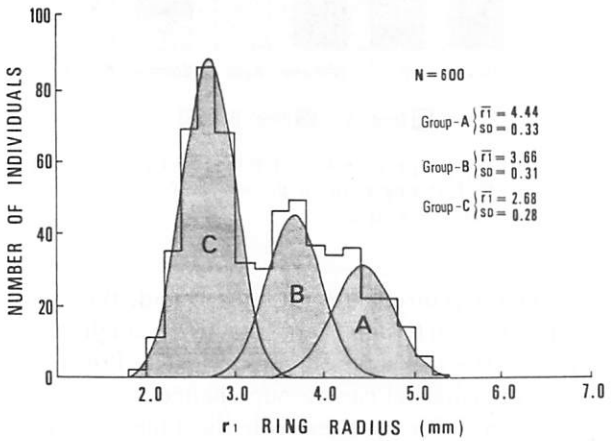

Figure 3. The composition of the first ring radius on the scales of 1-year-old red sea breams collected from 6 areas, with fitted normal curves of distribution in each group.

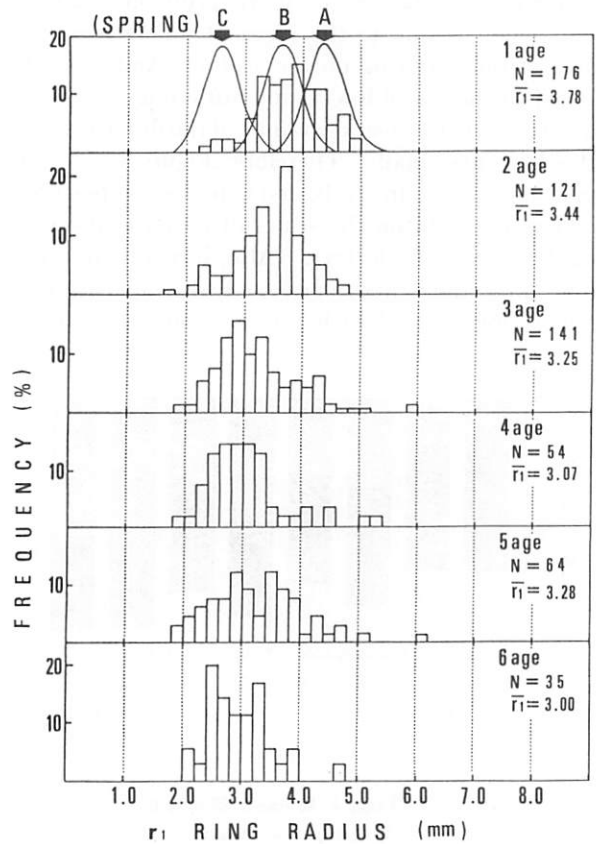

Figure 4. Frequency distribution of the radius of the first ring (r_1) on the scales of the red sea bream were caught off Kyoto Prefecture in spring.

b: 1 to 6-year-old fish in the sea off Kyoto Prefecture

The red sea breams are caught off Kyoto Prefecture twice a year in spring (April–June) and autumn (September–November). Figure 4 and 5 show the compositions of the first ring radii on the scales by the age of fish caught in spring and autumn. These reveal that the percentage occurrences of those with small first ring radius (Group C) increased as they get older. The disappearance of A and B Groups can be seen in the compositions of the 4 to 6-year-old fish. And the average of the first ring radius on the scales decreased with age.

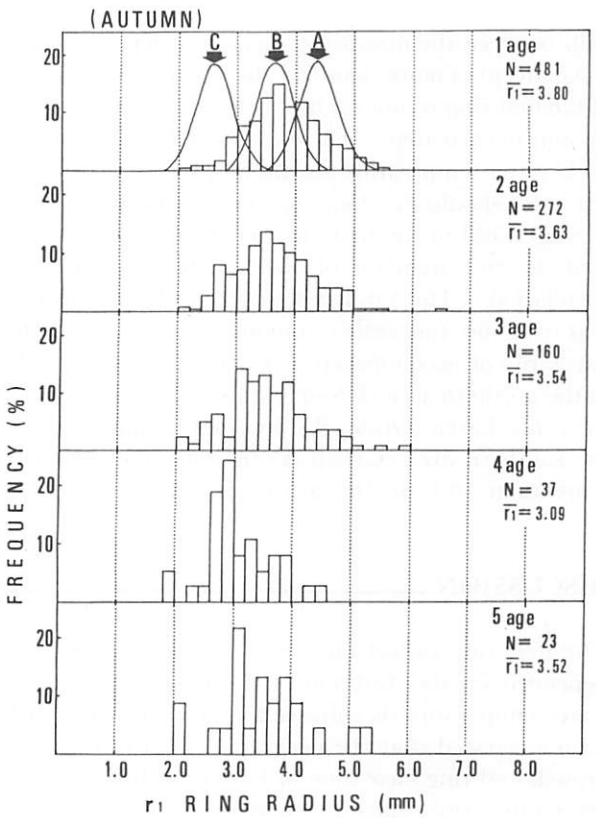

Figure 5. Frequency distribution of the radius of the first ring (r_1) on the scales of the red sea bream were caught off Kyoto Prefecture in autumn.

RELATIONSHIP BETWEEN THE FIRST RING RADIUS AND WATER TEMPERATURE

Generally, the differences in the growth mark on the scale, otolith and vertebral counts of fish are inclusively or indirectly related to water temperatures during early developments. The water temperature of more than 18°C is known as one of the better growth conditions for this species (Fujita 1969). Figure 6 shows the relation-

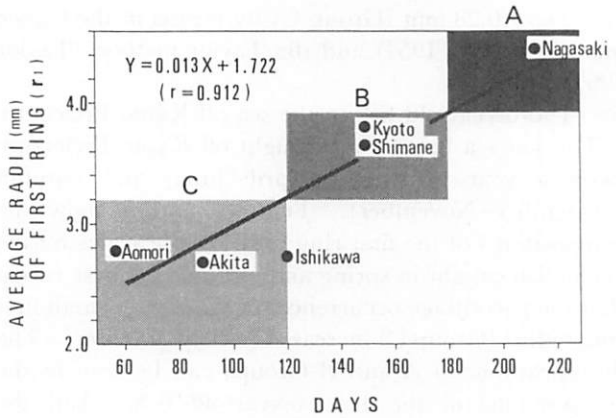

Figure 6. Relationship between the number of days in water temperature of more than 18°C in a year and average radii of first ring (r_1) on the scales of 1-year-old red sea breams in each area.

ship between the number of days in water temperature (50 m deep) of more than 18°C in a year and the average of the first ring radius on the scales of 1-year-old red sea breams in each area. The number of days in more than 18°C water temperature should be short in the northern sea and should be long in the southern sea. The average radii of the first ring on the scales correspond well to the number of days with a rather high correlation. The 3 different groups in the radius of the first ring on the scales can also be grouped into 3 categories of sea temperature, such as less than 120 days in the northern area (Group C), from 120 to 180 days in the central area (Group B), and more than 180 days in the southern area (Group A) in water temperature of more than 18°C in the Sea of Japan.

DISCUSSION

The first ring radius on the scales of red sea breams depended on the duration of the period in which the water temperature is suitable for their growth. It has been suggested that the geographical variations of the growth and ring formation of 1-year-old fish come from the temperature difference and reflect the metabolism difference of the fish in each water. Bilton and Messinger (1975) reported that temperature has been considered to be the main factor affecting the width of circulus intervals, because it profoundly affects somatic growth. Namely, the compositions of the first ring radii on the scales in each area reflect the differences in developmental rates of 0 age from area to area. Therefore utilizing these findings, the proportions of Groups A, B, and C in the composition of the first ring radii on the scales of 1-year-old red sea breams were calculated in the area under investigation (Figure 7). Group A is expected to appear prominently with a high percentage of 1-year-old fish in the southern sea off

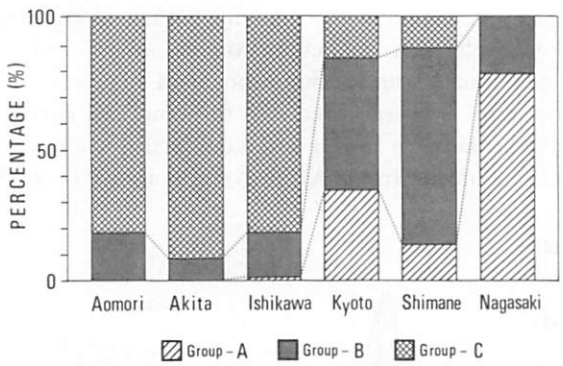

Figure 7. Calculated proportions of Groups(A~C) in the compositions of the first ring radii on the scales of 1-year-old red sea breams in the each area.

Nagasaki Prefecture. On the other hand, the 1-year-old fish belong to Group C and should be caught mostly from the northern sea off Aomori and Akita Prefectures. These proportions of each group change as the growth proceeds and also changes with the time of year?

Figure 4 and 5 reveal that the compositions of the first ring radii on the scales at the age of fish caught in the sea off Kyoto Prefecture referred to as "Lee's phenomenon". The causes of Lee's phenomenon have not been identified yet. However, it is probable that this phenomenon is due to the regional differences in the radius of the first ring on the scales. And if the fish hatched in the sea of low temperature migrate into the southern stock, the average radius of the first ring on the scales becomes small. Therefore, Figure 8 shows the proportions of Groups A, B, and C in the composition of the first ring radii on the scales of 1 to 4-year-old fish caught off Kyoto Prefecture. Also, it reveals that there are no large differences between two composistions of the first ring radii of each age in spring and autumn.

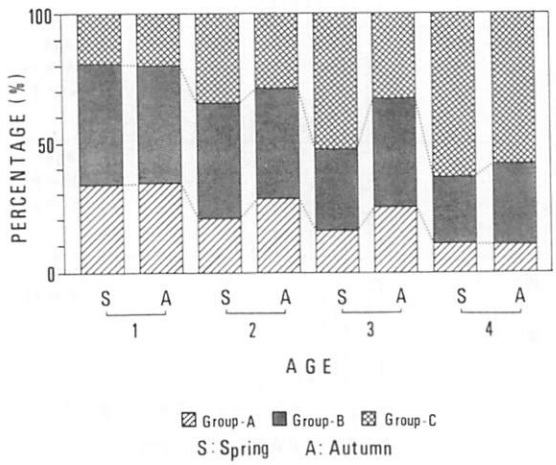

Figure 8. Calculated proportions of Groups(A~C) in the compositions of the first ring radii on the scales of red sea breams caught off Kyoto Prefecture classified by the age and season.

And the percentage occurrences of Group C increases with age and Group A decreases with age. Also differences in each composition between every autumn and spring in the following year cannot be disregarded. For example regarding fish 3 years old in autumn or older, the variation of the proportions of each group may refer to their sex maturation. Especially, matured red sea bream at about 4 years old (Munekiyo and Sobajima 1981) are largely composed of fish hacthed in the northern sea in the fishing ground off Kyoto Prefecture. And it is likely that their migrations in the Sea of Japan may be suggested from these results. The southern stock migrate into the central area of the Sea of Japan as they grow during the period from spring to autumn in every year, namely they move toward the northern sea. And the northern stock migrate into this area during the period from autumn to spring in the following year, then toward the southern sea.

Consequently, if the changes in the first ring radii on the scales of red sea breams in a given area reflect the extension of their habitat is associated with their growth, the differences in the first ring radii on the scales can be a key to the reality of their migration. And this can also be an important finding with regard to estimating the effects of the stock-laying of artificial seeds.

LITERATURE CITED

Akazaki, M. 1960. Growth and age of the sea bream, *Chrysophrys major*, caught in Wakasa Bay. Bull. Jpn. Soc. Sci. Fish. 26: 217–222. (In Japanese; English abstract).

Bilton, H. T,. and H. B. Messinger. 1975. Identification of major British Columbia and Alaska runs of age 1.2 and 1.3 sockeye from their scale characters. Bull. Int. N. Pacif. Fish. Comm. 32: 109–129.

Cassie, R. M. 1954. Some uses of probability paper in the analysis of size frequency distributions. Aust. J. Mar. Freshw Res. 5: 513–522.

Fujita, S. 1969. Culture and propagation of sea breams. Bull. Jpn. Soc. Sci. Fish. 35: 583–586. (In Japanese; English abstract).

Munekiyo, M,. and N.Sobajima. 1981. Migration of red sea bream around the sea off Kyoto Prefecture. Bull. Kyoto Ocea. Fish. Sci. 5: 1–16. (In Japanese; English abstract).

Murakami, S,. and K. Okada. 1967. Studies on the fishery biology of the sea brem, *Chrysophrys major* in the East China and the Yellow Seas-III. Bull. Seikai Reg. Res. Lab. 35: 23–40. (In Japanese; English abstract).

Taylor, B. J. R. 1965. The analysis of polymodal frequency distributions. J. Anim. Ecol. 34: 445–452.

Bull. Natl. Res. Inst. Aquacult., Suppl. 2: 105-114 (1996)

STUDY ON RESOURCES, ECOLOGY, MANAGEMENT AND AQUACULTURE OF JAPANESE FLOUNDER *PARALICHTHYS OLIVACEUS*, OFF THE COAST OF NIIGATA PREFECTURE

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ABSTRACT

A series of researches on the ecology of juvenile and young flounder have been carried out since 1981 in the estuaries and their adjacent waters in the area of Iwafune, northern district of Niigata Prefecture. Through these researches, various information has been acquired on fishery ecology of flounder including their biological characteristics (Kato 1986), population dynamics and life history (Kato 1985). In the area of Iwafune, flounder and dabs have been caught with a small scale otter trawl, "Itabikiami". Due to the small mesh size in the cod end (51 mm) of the fishing gear, a large number of juvenile flounders have been wasted. In addition to this irrational catch, overfishing of young flounders every year decreased the annual catch in 1987 to 207 metric ton, which is less than half of the 1975–1985 catch levels.

As a result of the sharp reduction in 1987, intense researches have been conducted especially with the implementation of three strategies: execution of a fisheries management program, appropriate release of reared juveniles, and realization of a nature preserve by the settlement of artificial reefs. Two important keys have been found at present study to promote stock enhancement of Japanese flounder. The primary finding is a possibility to predict the number of recruits. The relation of strong regression between the density of settled juveniles and number of recruits has become possible to predict the number of one year flounders. Therefore, this prediction can minimize overfishing and determine the optimal catch level. The other step is to improve their survival rate higher. We have to make some propositions for this purpose such as increasing mesh size of the cod end to 83 mm, establishing regulations to protect the area and to decrease catch rate, and correspodent release with the appropriate level of recruits.

INTRODUCTION

In the past 10 years many fishery problems have surfaced. It is my premise that three strategies for flounder stock must be enhanced. The first problem is to establish regulations regarding stock management. And it's important to prompt fishermen to keep the regulations. To suggest practicable regulations, we have surveyed the fishery ecology of flounder and flatfishes since 1982 (Kato 1985, 1992).

Our primary proposal regarding the regulations are minimum limited length which are based on the biological minimum size, an enlargement of a small size mesh of cod end, and a drastic retrenchment of fishing effort.

The secondary problem is an estimation of stocking effectiveness by a sampling survey of commercial landings. It's important that results such as an investment effort is made public.

The final strategy is an effective settlement of artificial reefs. This task would enable our proposal to enhance the flounder stock level in a sense of the resting zone.

FISHING GROUND AND THE CHANGES OF ANNUAL LANDINGS

Niigata Prefecture and our research field are showed on Figure 1. Niigata Prefecture is situated in the central part of the Japanese mainland, Honshu. We have the longest river, Shinano River, in Niigata Prefecture. The Shinano River and some other rivers have carried a large quantity of sandy mud into this area for several thousand years. Sandy mud areas comprise about 6,056 km^2 of the continental shelf in the northern district. The research areas cover about 66.7% of a whole square on the continental shelf, along the coast of Honshu, Niigata Prefecture. Japanese flounder

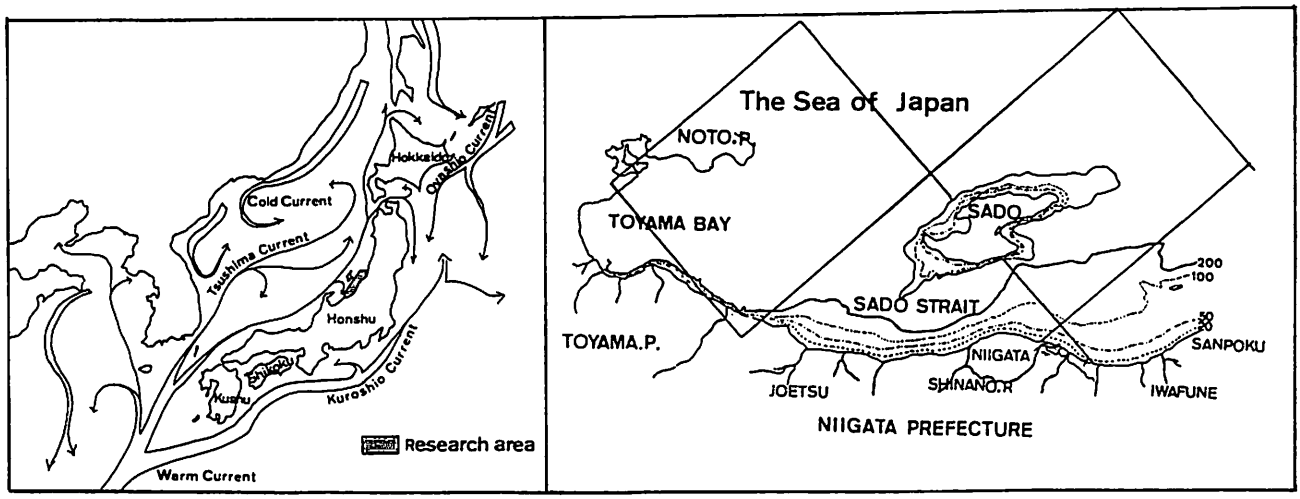


Figure 1. Schematic view of sea currents around Japan and research areas of Niigata Prefecture in the Honshu coast.

'Hirame' and other flatfishes have been caught by smallscale otter trawl fishery "Itabikiami" for about 20 years on this fishing ground. Thus 'Itabikiami' has been marked as the main target for fisheries management. Four important species of the flatfish inhabit the continental ground in the north coast of Niigata Prefecture. These are Japanese flounder 'Hirame' (*Paralichthys olivaceus*) and dabs: brown sole (*Limanda herzensteini*), marbled sole (*Tanakius kitaharai*) and roundnose flounder (*Eopsetta grigorjewi*). The small scale otter trawl fishery is so efficient that the catch of most flatfishes has been reduced within seven or eight years since their year of operation.

The first operation for 'Itabikiami' was in 1963. In 1965, this fishing method was first regulated with a license-limit to the northern coast of Honshu, in Niigata Prefecture. After several years this regulation was extended to cover the northwestern areas of Sado Island too.

The 1972 annual catch of Japanese flounder in

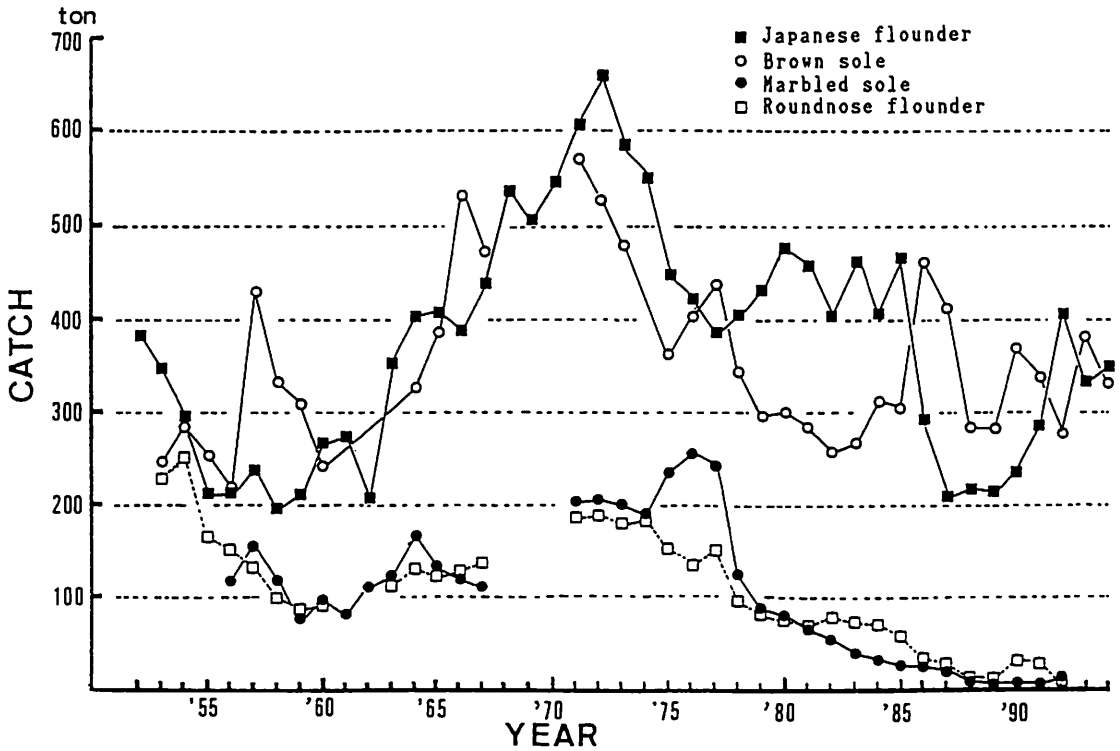


Figure 2. Changes of the annual landings of four important commercial flat fishes off the Honshu coast of Niigata Precture.

Honshu coast of Niigat Prefecture yielded a record stet 658 t. However, the cod end's mesh size of the "Itabikiami" is so small that a large number of flounder juveniles and young fishes have been devastated after extreme irrational catch. In 1977, extreme continuous overfishing has decresed the annual catch to below 400 t levels. After this decrease, the annual catch had been fluctuated at 400 t levels for eight years. Unexpectedly in 1986, the annual catch was reduced to 230 t; in 1987 the catch was reduced to 200 t (Figure 2).

The fall in the catch of Japanese flounder impacted many fishermen greatly similar to the incident of 'oil shock' in Japan. I did call it 'Hirame shock'. Some 'Itabikiami' fishermen in Niigata City made an intense effort to organize a movement to protect the flounder stock against an irrational catch in 1986. Until quite recently, we have issued a stern warning to the "Itabikiami" fishermen.

As a result into consideration, the administrative agency, the institutional service and the fishery industry have drawn up a guiding principle (Kato 1993).

BIOLOGICAL AND ECOLOGICAL SYNOPSIS OF JAPANESE FLOUNDER IN NIIGATA COAST

GROWTH OF JAPANESE FLOUNDER

There is a difference in growth between female and male flounders. Similar difference is observed in dabs too. Usually, the growth of female flounders surpasses the male. In the coast of Niigata, female flounders grow to 19 cm in one year, 30 cm in two years, 40 cm in three years, and 49 cm in four years (Kato et al. 1987).

The present fishing regulation size is 25 cm or above in length (Figure 3). Female flounders grow to 25 cm in 17 months. Growth of male flounders is slower to females by two months. Until now, we have'nt sampled specimens of a male flounder more than 55 cm in total length. The past maximum record of a female flounder is 100 cm and 15 kgr and the age was 12 years.

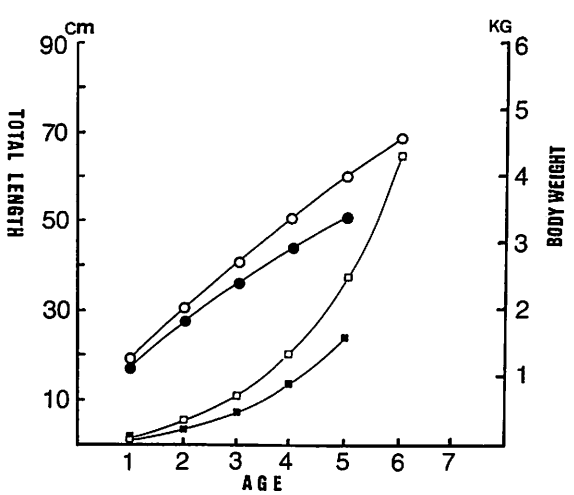


Figure 3. Growth curve of Japanese flounder in the Honshu coast of Niigata Prefecture.

SPAWNING SEASON OF JAPANESE FLOUNDER ON THE HONSHU COAST IN THE SEA OF JAPAN

A relationship between the isothermal line of 15°C and spawning season in the Japan Sea coast is shown in Figure 4. The northward of isothermal line is influenced by the Tsushima warm current. Stel on the coast of Niigata comes into season from the middle of May to the middle of June, according to the increase of females' gonad somatic index (GSI) (Kato et al. 1987). There is a difference of three months or above in the spawning season between the far west in the Honshu and the far north (Kato 1987). Under microscopic observation of flounder eggs, its average diameter is about 1 mm. And in their frequency distribution, three modes of diameter was observed. Therfore their spowning times in season are estimated more than three times or above.

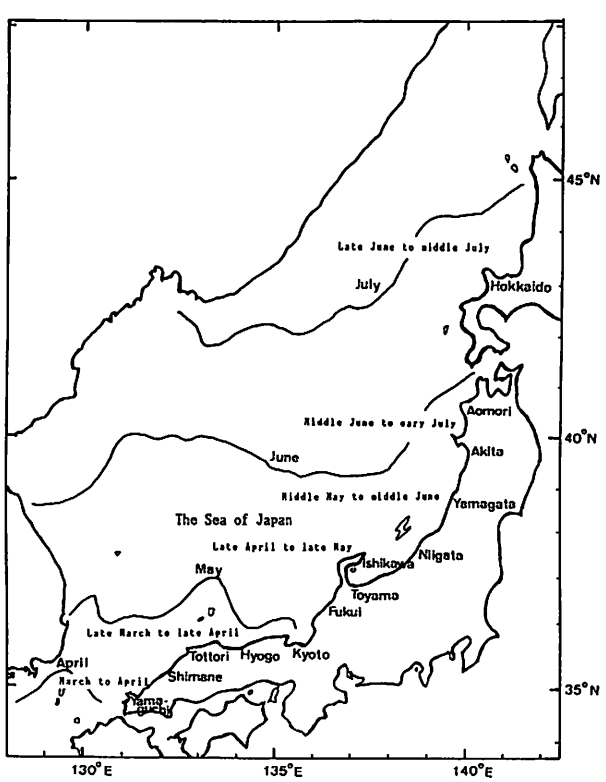


Figure 4. A relationship between the isothermal line of 15 °C and spawning season of Japanese flounder in the Japan Sea coast.

SEX RATIO, MATURITY AND NUMBER OF EGGS OF JAPANESE FLOUNDER

The sex ratio, maturity, and number of eggs of Japanese flounder are shown in Figure 5 and Table 1. Through some researches, it has been observed that the sex ratio of 0 year flounders below 25 cm in TL was fluctuated every year. But these reasons why their sex ratio fluctuate are not concluded. The sex ratio of three years' in favor of females is 67 to 33, in four years' 87 to 13, in five years' 91 to 9 and above six years' are all females. Maturity of three years' flounders are estimated about 50%. It becomes 100% more than four years'. There's a regression between ages and number of eggs. As a result of the microscopic observation, it was found that they keep about 1,170,000 ovarian oocytesin three years, 1,720,000 in four years, and 2,340,000 in five years (Kato 1987).

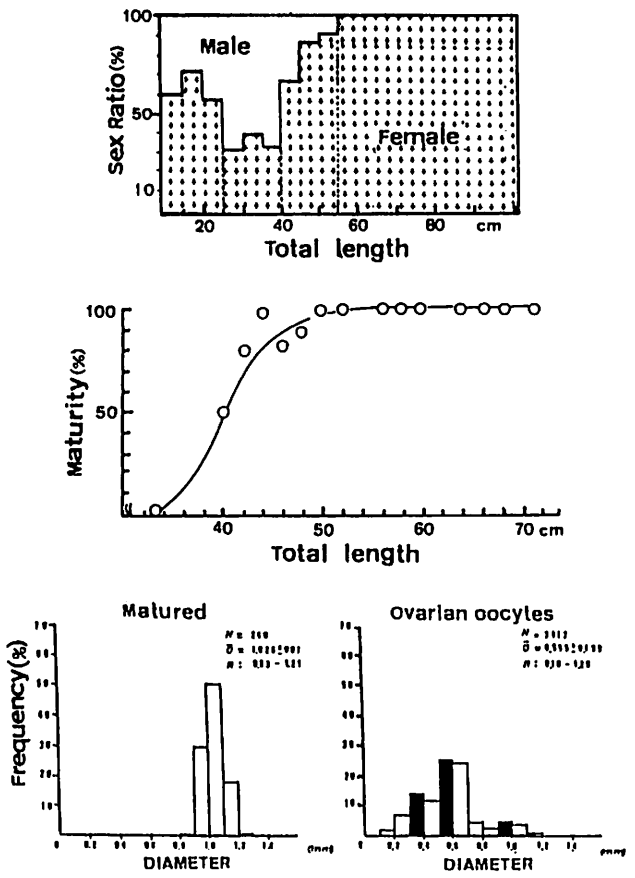


Figure 5. Sex ratio, maturity in each total length, and frequency of the diameter in ovarian oocytes of Japanese flounder

Table 1. Number of ovarian oocytes (N.O.O) in each age of Japanese flounder in Niigata coast

Ages	Sex ratio	N.O.O(*10^3)
3	0.67	1,156
4	0.87	1,720
5	0.91	2,340
6	1.00	3,010
7	1.00	3,723
8	1.00	4,476
9	1.00	5,266

N.O.O= {25.39×(Age)^1.38} ×10^4

TRANSPORT OF FLOUNDER EGGS AND LARVAE WITH THE TSUSHIMA CURRENT

Fertilized eggs and pelagic larvae of Japanese flounder are transported with the sea current. The fertilized eggs take about three days for hatching and larvae open their mouth within three additional days. Then they spend a pelagic life for a month. It seems to survive at least for 11 days above without a eating based on the result of a breeding experiment of settled larvae (Tanaka 1988). Next, Niigata Prefectural Fisheries Experimental Station (NPFES) had released a sealed postcard with a vinyl sheet like a child's identification tag at a spawning area off the southwestern coast of Niigata Prefecture in 1974–1976 (NPFES 1985). In fact our released postcards had reached Ibaragi Prefectural coast of the Pacific Ocean and Okhotsk sea coast in Hokkaido. Based on the test of starvation, I firmly believe that both the eggs and larvae will survive enough if they are transported by the sea current distant

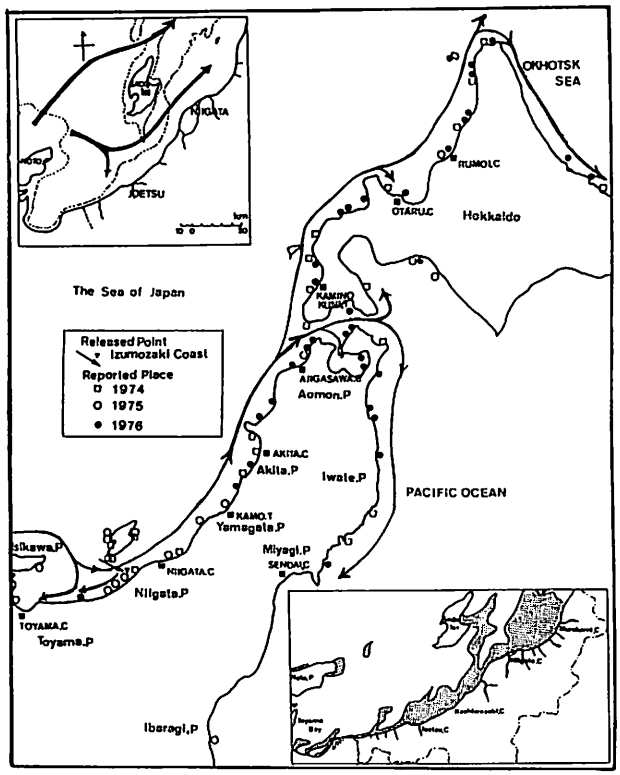


Figure 6. Schematic view of transporting of fertilized eggs and larvae, based on the result of experimental release of vinyl coverd postcards with Tsushima warm current.

estuary waters. In Figure 6, coastal streams of Niigata Prefecture are shown to increase the turbidity of the coastal waters (NPFES 1985). I am certain that these turbid waters act as a limiting factor on the distribution of the eggs.

FEEDING HABITS AND DIURNAL CHANGES OF JUVENILES AND YOUNG FLOUNDERS

Pelagic flounder larvae metamorphose at about 13 mm in TL. Then they settle on the sea bottom of at a depth of 15–20 m. The season of flounder settlement on the seabed in the northern coast of Niigata Prefecture is estimated in every late June. Pelagic flounder larvae in Japan Sea waters feed on *Copepoda* and its eggs, *Penilia, Oikopleura, Evadne* and other zooplankton (Kuwabara and Suzuki 1982). Especially, they feed on *Paracalnus* selectively. *Mysidacea* is the main diet of eary demersal stage flounder juveniles in the coastal waters of Niigata Prefecture (Figure 7). The piscivorous feeding habit occurs at 7 to 12 cm in TL (Kato 1985). The demersal flounder juveniles move from 3–6 m deep bottom down to depth 20–50 m in August at 15 cm in TL. The time of active feeding for flounder juveniles is immediately after sunrise and before sunset, and they do not feed at night (Figure 8). Thereafter, they migrate seasonally between 130 m deep and shallow waters. (Kato 1987)

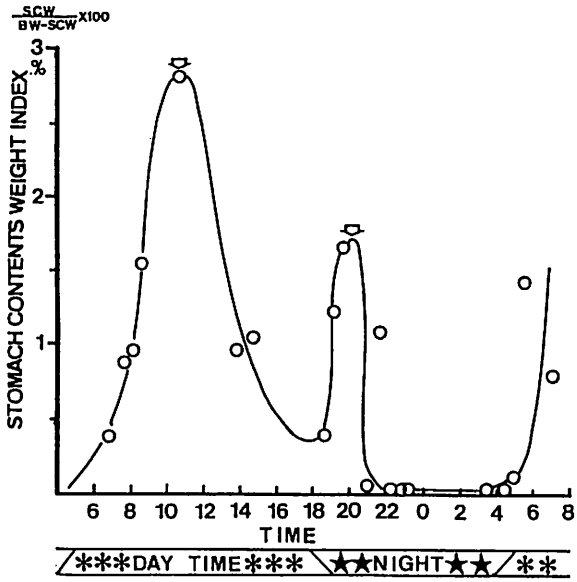


Figure 8. Diurnal changes in stomach contents weight index of flounder juveniles in north coast of Niigata Prefecture.

SEASONAL CHANGES IN ABUNDANCE OF FLOUNDER JUVENILES AT THE SANDY SEA BOTTOM OF THE SENAMI COAST

After settlement on the sea bottom in early June, flounder juveniles migrate gradually into shallow waters

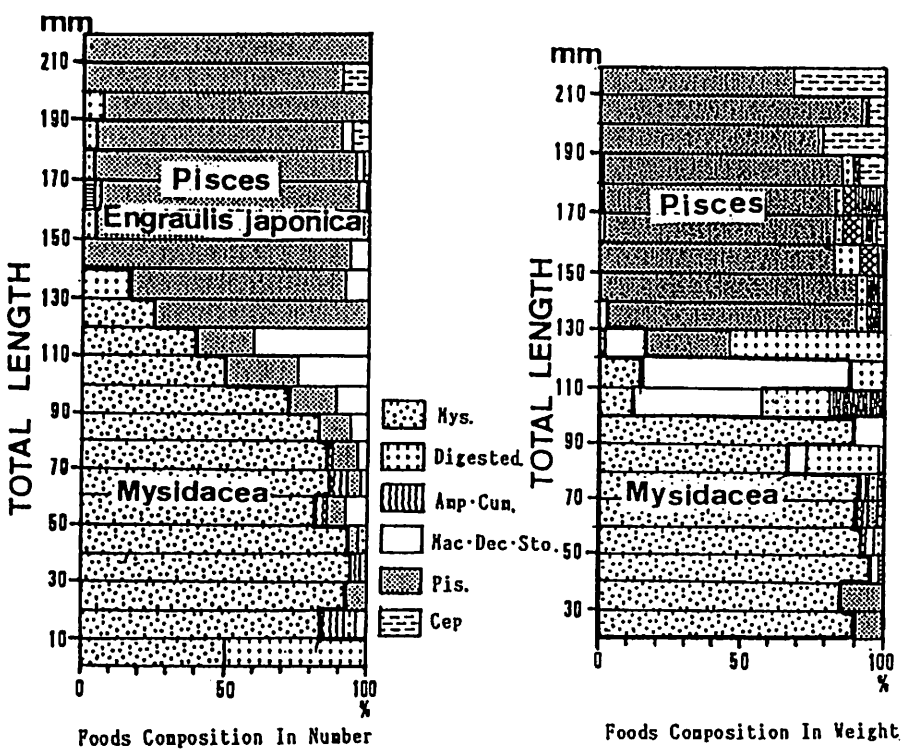


Figure 7. Changes of stomach contents with length for juveniles and young flounders sampled in north coast of Niigata Prefecture.

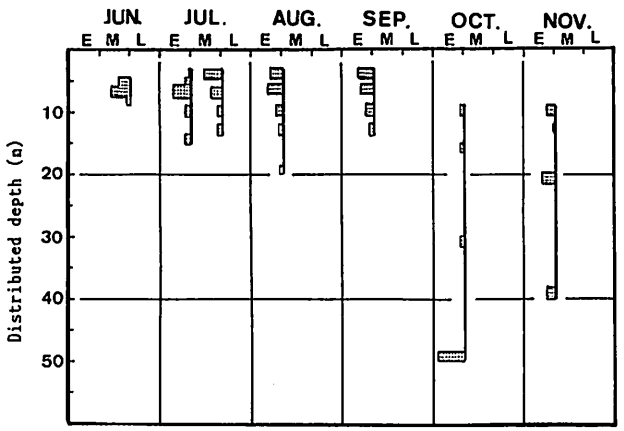


Figure 9. Seasonal changes in distributed depth of juveniles and young flounders in north coast of Niigata Prefecture.

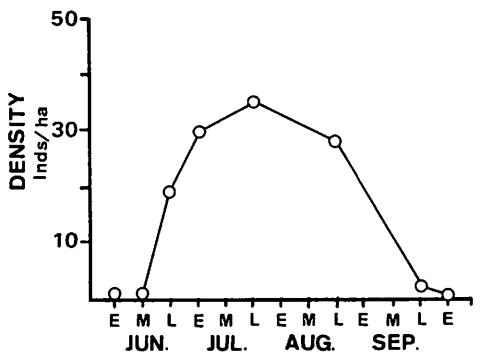


Figure 10. Seasonal changes in abundance of flounder juveniles on the sandy sea bottom of Senami coast within the 3–21 m deep.

in the depth of 3–6 m (Figure 9). In the Senami coast, flounder juveniles are most abundant in late July to early August (Figure 10). They depend on abundance of the large size *Mysidacea* for their growth and survival. Almost flounder juveniles show piscivorous habit at 13 cm above in TL. Their main diet is an anchovy larva and a juvenile (*Engraulis japonica*). I suppose that they migrate between 10 and 50 m deep water accompanying shoals of anchovies day by day from October to November (Kato 1985, 1987).

RESOURCES DYNAMICS AND MANAGEMENT

Nowadays, we are required to produce scientific data and to establish wild stock assessment in our fishery research. Since the extreamly reduced year in 1986 and 1987, fishermen have began to act for restoration of reduced flounder stock in the Honshu coast of Niigata Prefecture. Now, I'll introduce flounder fishery and population dynamics in the northern coast of Niigata Prefecture.

MIGRATION OF YOUNG FLOUNDERS

In Figure 11, the migration of young flounders from each coast of the North Japan Sea by tagging is shown. Some of the young flounders migrate southwest (Nashida, Hasegawa, and Kato 1986), but in the case of Niigata Prefecture, most of them were recaptured within

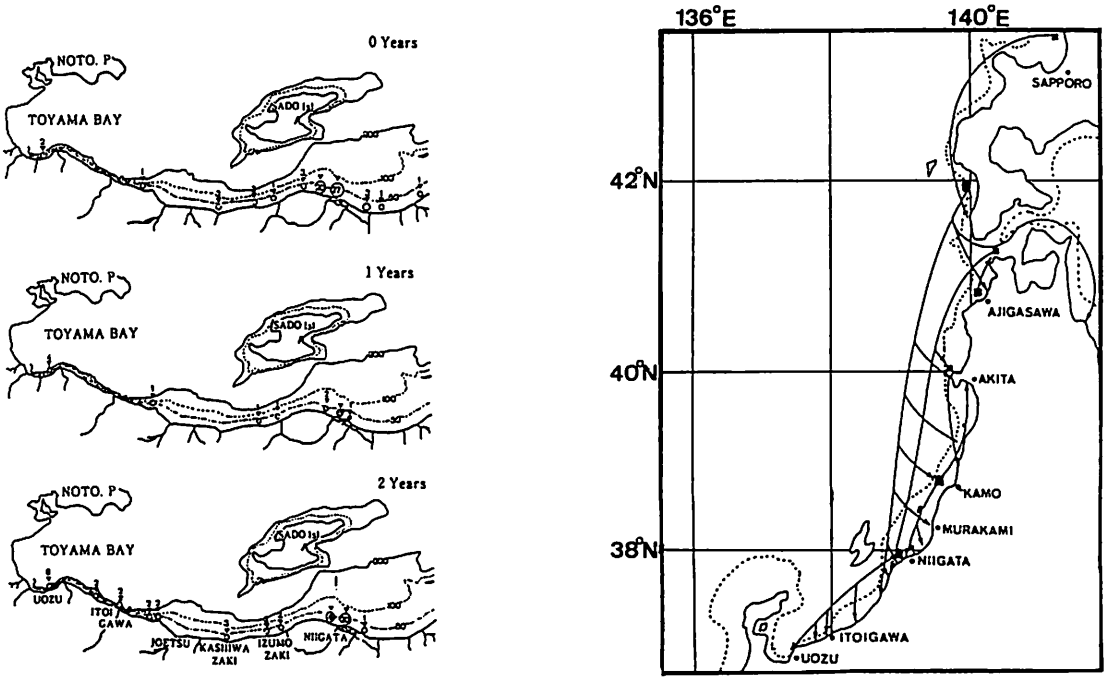


Figure 11. Migration of young flounders based on tagging experiments in north Japan Sea coast.

the north coastal areas. I have believed a working hypothesis that most of the young flounders migrate only within a ceretain area through their lifetime. Some of the eggs and pelagic larvae are transported by the sea current from the east coast of Toyama Prefecture to South Hokkaido coast, thereafter they settle on the shallow and brackish waters of estuary. Some of the transported and settled young flounders will come back to their spawning areas.

RELATION BETWEEN DISTRIBUTION DENSITY SETTLED JUVENILES AND RECRUITS IN ZERO YEAR FLOUNDERS

Since 1981 I have surveyd the density of flounder juveniles in the esturies of the Miomote River and their adjacent waters "Senami coast" in the north coast of Niigata Prefecture. Recruits of 0 year flounder have been calculated by measurd data in a fisherman's market at Iwafune port. A regression of recruits on the density of flounder juveniles which had an impact on me has been studied for 13 years (Figure 12). The working hypothesis that I have mentioned in the former chapter enables us to estimate the stock recruits of the entire north coast in Niigata Prefecture.

AGE COMPOSITION OF THE COMMERCIAL CATCH IN NORTH COAST OF NIIGATA PREFECTURE AND SOME ISSUES OF RECRUITS OVERFISHING

Mode of catch in ages from 1979 to 1983 was studied in year classes. Primary overfishing in 0 year class has occurred in 1984. In 1985, subsequent overfished year class was 1984 year class. Moreover, secondary overfishing in 0 year class has occurred in 1986 (Figure 13).

Recruits of 1985 and 1986 year class were diminished less than half of the year before (See Figure 12). These occurrences for four years in succession account for real reason that "Hirame shock" stel occurred in 1987. I believe it is too difficult to prevent overfishing of recruits by the mesh size of cod end now in use.

RELATION BETWEEN SELECTIVITY RATE AND SELECTIVITY COEFFICIENT OF THE COD ENDS MESH SIZE IN SMALL OTTER TRAWL FISHERY

Young flounders migrate between 40–60 m deep all the year round. On the other hand, in spite of changing a fishing target and trawling depth seasonally, bycatch is inevitable so long as we continue to use the small scale otter trawl cod end. We have examined fishing selectivity using five mesh size of the cod end for several years in the north coast of Niigata Prefecture (Figure 14). We have found that increasing mesh size of the cod end up to 82.7 mm from the present 50.5 mm is the best selection (NPFES 1992).

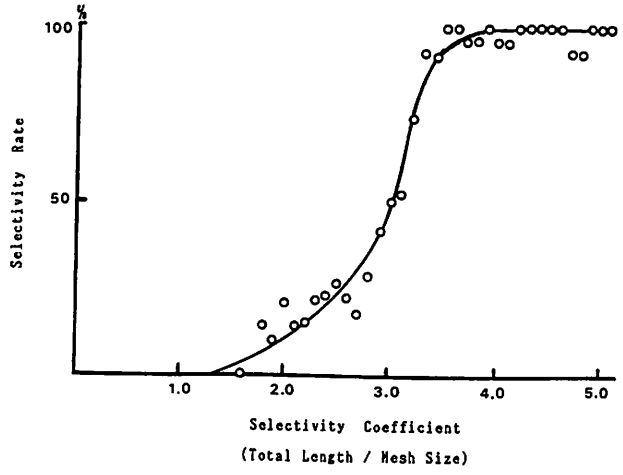
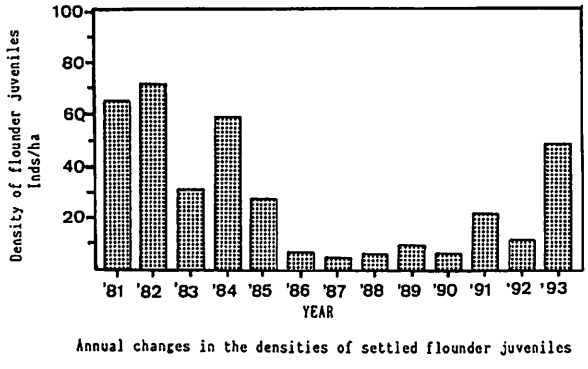


Figure 14. Relation between selectivity rate and selectivity coefficient of the cod end's mesh size in small scale otter trawl fishery.



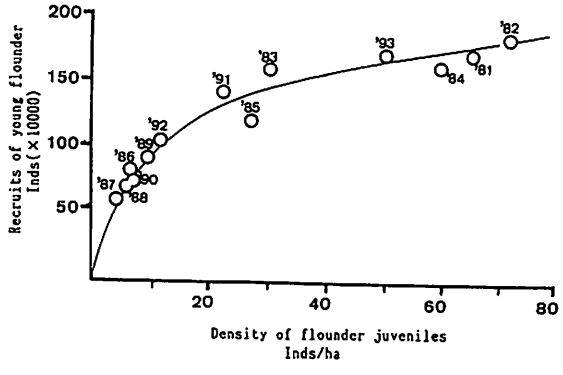


Figure 12. A regression between annual densities of settled juveniles and recruits of young flounder in north coastal region of Niigata Prefecture.

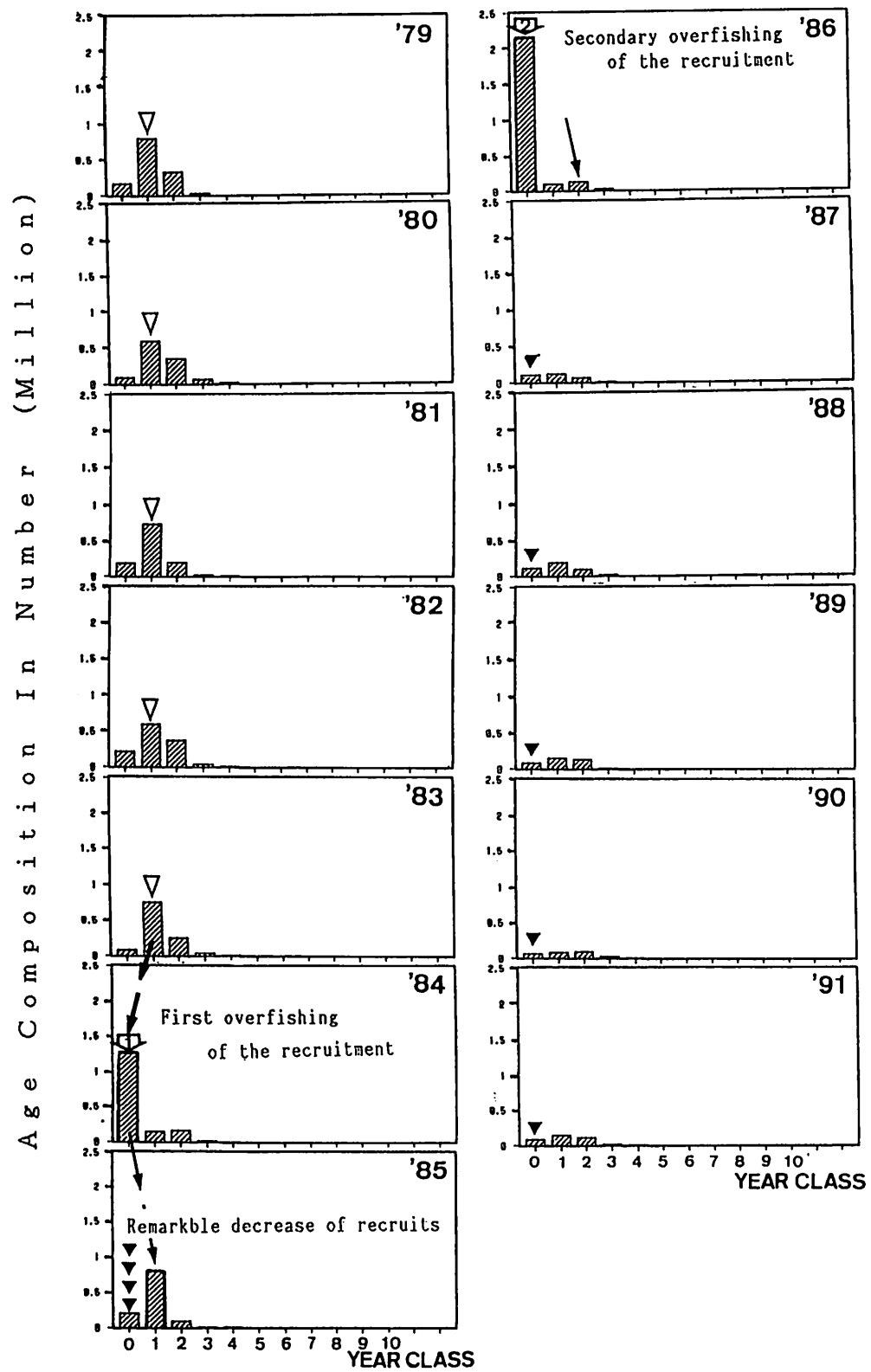


Figure 13. Changes in the age composition of the commercial catch in north coast of Niigata Prefecture.

STOCKING THROUGH THE FARMING FISHERY

RECAPTURED REGION OF REARED FLOUNDER JUVENILES

Particular characteristics in reared juvenile flounders are a hypermelanosis on the blind body side. Market research in Iwafune port based on the hypermelanosis have been put practiced since 1985.

By the experimental release of reared flounder juveniles, we have made their movement, growth, and recapture rate distinct. Experimental release has been done for five years since 1985 (NPFFC 1988).

According to the result of 1985 year's release, about 99% of the reared juvenile and young flounders were recaptured within 50 km of the released point in the northcoast of Niigata Prefecture (Figure 15). This result supported my working hypothesis concerned with a separation of subpopulation.

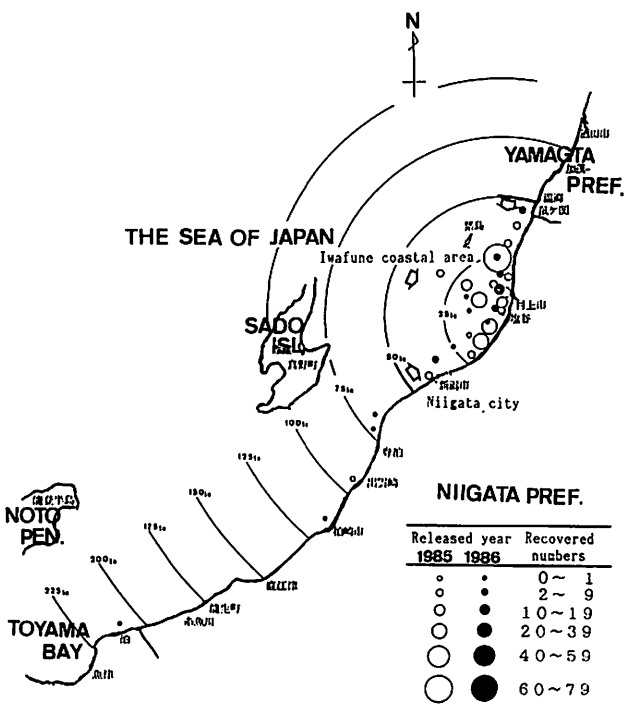
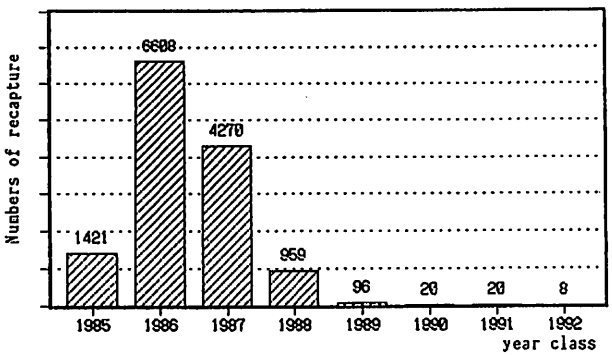


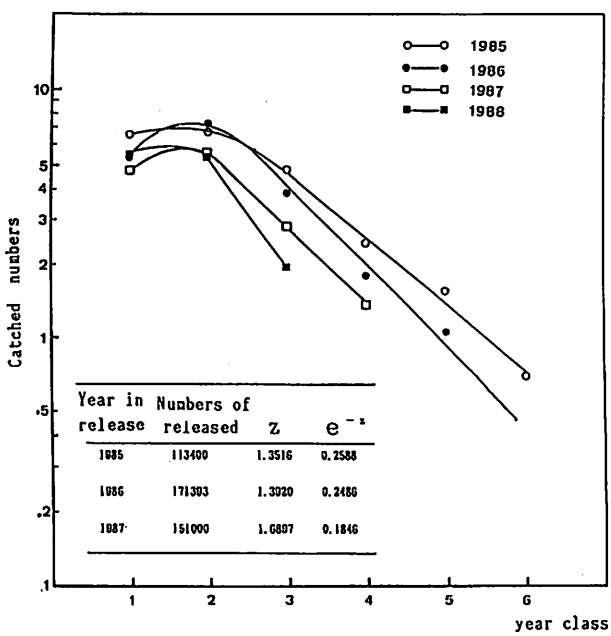
Figure 15. Recaptured region of reared flounder juveniles released off Iwafune coast in 1985.

ESTIMATES OF STOCKING EFFECTIVENESS BY A SAMPLING SURVEY OF COMMERCIAL LANDINGS

An estimated recapture rate of the release in 1985 is 13% accumulative (Kato unpublished). And their recapture time continued for eight years. Their survival rate is 25.9% in 1985 release, 24.9% in 1986



Annual recapture of reared flounder released in 1985.



Estimates of a survival rate of reared flounders released in the Iwafune coast from 1985 to 1988.

Figure 16. Estimation of stocking effectiveness by a sampling survey of commercial landings.

release (Figure 16). The density of wild flounder juveniles in 1985 decreasd down to 14.3% of the 1984.

After this, a decline tendency of the juvenile density continued for 6 years from 1985–1990. A decrease of the recruits which is caused by a decline of the density had an good influence on their survival to reared juveniles. I cannot estimate the carrying capacity in the north coast of Niigata Prefecture concerned with wild and reared flounder juveniles. But we should explicate a sustainable number of release that they prompt reared flounder juveniles to survive enough from now on.

CONCLUSION

In 1992, We have formed an experimental ground with some types of artificial reefs sank at 29 m deep bottom

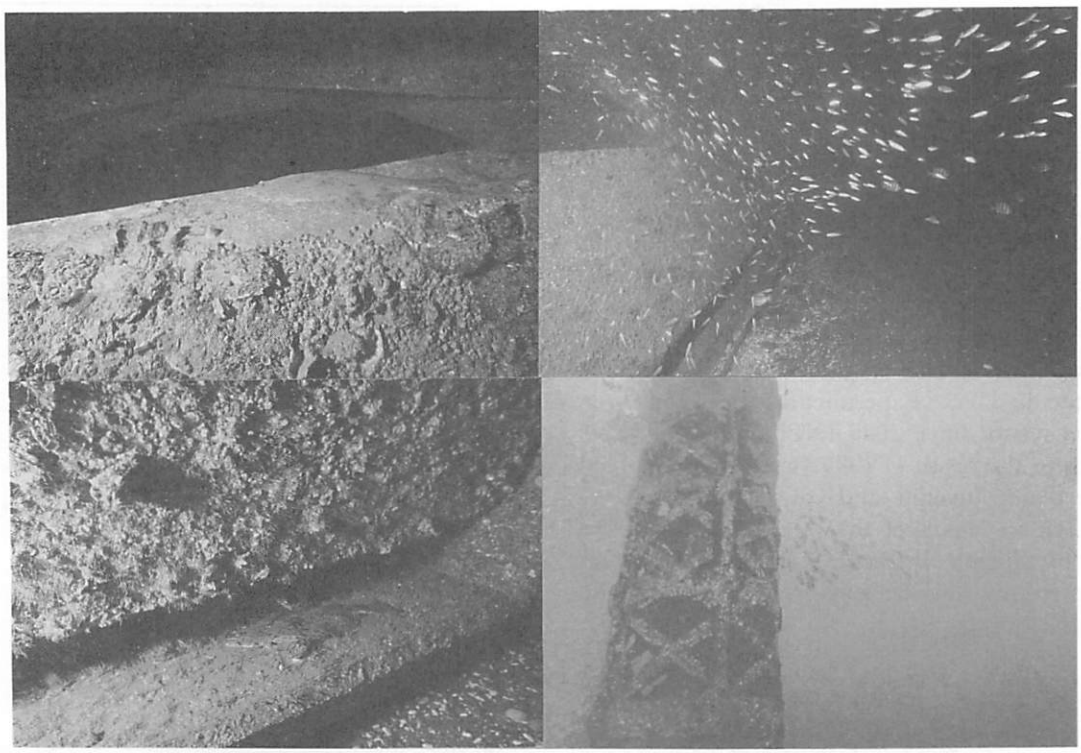


Figure 17. Photographs of a settled young flounder on the artificial reefs and their small feeding fishes gathered around reefs arranged in stud waters.

off the coast of Iwafune (Figure 17). This point is in the regulated area where wild young flounder inhabit year round. We have surveyed for three years at this study area using remotely operated vehicle (R.O.V), self contained under water breathing apparatus (SCUBA) and trawl net. We have to clear two problems in this survey. One of them is to increase the growth and survival rate of juveniles and young flounders, and the other is to prevent fishermen from irrational and disobedient fishing operation by 'Itabikiami'. Within five years, some typical artificial reefs would be constructed on the bottom in this study area. We would expect the enhancement of flounder stock recruits and increased catch in this area.

LITERATURE CITED

Kato, K. 1985. Distribution and food habit of Japanese flounder juveniles in north sea water region of Niigata Prefecture, Marine Ranching Program, Progress Report Japanese flounder and halibut (I), Seikai Reg. Fish. Res. Lab., pp. 93–105. (In Japanese)

Kato, K. 1986. Migration and biological characteristics of Japanese flounder in north sea water region of Niigata Prefecture, Marine Ranching Program, Progress Report flounder and halibut (II), Seikai Reg. Fish. Res. Lab., pp. 121–133. (In Japanese)

Kato, K. 1987. Ecological stydies on Japanese flounder in Niigata coastal waters. Fisheries Technology and Management., March, pp. 46–54. (In Japanese)

Kato., K, W. Anzawa and K, Nashida. 1987. Study on Resources Management of Flounder (Paralichthys olivaceus) in the Northern Coast of Niigata. II. Niigata Fisheries Experimental Station Bull., pp. 42–59. (In Japanese)

Kato, K. 1992. Study on resources dynamics of a Dab (Limanda herzensteini) in coastal waters of Niigata Prefecture. Proceedings of North Japan Scientific Council on the Fisheries Resources., Vol, 25, pp. 27–49. (In Japanese)

Kato, K. 1993. Resources management of Japanese flounder in Niigata Prefecture. A manual of fisheries management of Japanese flounder. The Association of Fisheries Cooperative Society in Japan. Series of manuals, No 3, pp. 10–22. (In Japanee)

Kuwabara, A and Suzuki, S. 1982. Vertical distribution and feeding of a flounder Paralichtys olivaceus larva. Nippon Suisan Gakkaishi., 48, pp. 1375–1381.

Niigata Prefectural Fisheries Experimental Station., 1985. General report on studies of artificial reefs and its concerns in Niigata coastal waters., pp. 53–57. (In Japanese)

Nashida, K, Hasegawa, S and Kato, K. 1986. The Present Status of Small Otter Trawl Fishery and Its Management in the Coastal Waters of Northern Niigata Prefecture., Proceedings of the Japan Scientific Council on the Fisheries Resources., No. 25, pp. 117–137.

N.P.F.E.S., 1992. Selectivity of the cod end mesh size in the small otter trawl fishery. (In paper)

Tanaka, M. 1988. Settlement and mortality of flounder (Paralichthys olivaceus) larvae and juveniles in Sishiki Bay, Marine Ranching Program, Progress Report Japanese flounder and Halibut (3), Seikai Reg. Fish. Res. Lab., pp. 1–16. (In Japanese)

Bull. Natl. Res. Inst. Aquacult., Suppl. 2: 115-118 (1996)

WATERSHED IMPACTS ON CALIFORNIA SALMON POPULATIONS

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ABSTRACT

Salmon populations that supported commercial fisheries since the 1850s have seriously declined throughout the state of California. Habitat degradation that began in the 1800s with hydraulic mining, continues today as a result of agricultural water diversions, timber harvest, hydroelectric dams, gravel mining, cattle grazing, loss of riparian vegetation, and water pollution. To mitigate for some of this habitat loss, salmon aquaculture developed as a system of state and federal hatcheries producing smolts for release in a stock enhancement program. This hatchery program continues today but has been unable to overcome the cumulative impacts of watershed activities that have resulted in drastically reduced salmon populations. Some habitat improvement is beginning to occur, through cooperation between landowners, regulatory agencies and the public, under the mandates of the Federal Clean Water Act and the Endangered Species Act.

INTRODUCTION

Salmon populations that supported significant commercial fisheries since the 1850s have seriously declined throughout the state of California. Habitat degradation that began in the 1800s with hydraulic mining continues today as a result of agricultural water diversions, timber harvest, hydroelectric dams, gravel mining, cattle grazing, and water pollution from industrial and residential development. While no individual factor is responsible for declining fish populations, land use practices associated with resource use and extraction have resulted in erosion, elevated temperatures, habitat reduction, and reduced water flows that have seriously impacted many salmon runs.

In 1991, the American Fisheries Society identified 214 stocks of salmonids in California, Oregon, and Washington that are of special concern, or face a moderate to high risk of extinction (*Nehlsen et al., 1991*). Of these, 39 stocks are in California and the Sacramento River winter run chinook have been listed as endangered, while the Russian River pink salmon are believed to be extinct. Nineteen stocks are at high risk of extinction, twelve are considered at moderate risk, and an additional six species are of special concern.

LAND USE PRACTICES

The earliest land use practices that significantly degraded salmon habitat in California resulted from gold mining activities undertaken following the gold rush in the middle of the 19th century. Placer mining, with hydraulic cannons for dredging, and washing sediments to extract gold nuggets, resulted in serious erosion and sedimentation. This destroyed spawning and nursery habitat in hundreds of miles of tributary streams in the Sierra Nevada foothills. Hydraulic mining in the Klamath National Forest's Salmon River from 1850 to 1930 deposited an estimated 12.1 million m^3 of sediment in the river bed. The influx of people during and after the gold rush increased agricultural, residential, and commercial uses in the watersheds. Current gravel mining practices continue to alter stream flow and substrate. The resulting impacts of these activities have imperiled many salmon stocks in California more than a century later.

As the human population in the west increased, land was increasingly used for crop production and livestock. The earliest direct impact of agriculture on salmon stocks was through the harvest of salmon during spawning runs and their subsequent use as fertilizer for crops. Early crop production resulted in severe erosion and removal of riparian cover that provided shading for streams. This erosion and sedimentation eliminated many spawning areas and filled pools that had provided nursery habitat. Loss of riparian cover increased sunlight penetration and raised temperatures, while eliminating large woody debris that provided cover and nutrients to support the food chain.

Cattle ranching operations cleared millions of hectares of land which further exacerbated problems associated with erosion and loss of riparian cover. Pennsylvania researchers identified significant differences in erosion and instream fauna in a comparison of grazed and ungrazed sub-basins in the Spring Creek watershed (Wohl and Carline 1995). Annual sediment loads from two streams with riparian pastures were 52 and 120% higher than those measured in the ungrazed Spring Creek basin. The pastoral streams, Slab Cabin Run and Cedar Run, had densities of benthic macroinvertebrates less than one-half those measured in ungrazed Spring Creek. Densities of brown trout in Spring Creek were five times greater than found in Cedar Run, and 68 times greater than those in Slab Cabin Run. In addition to erosion and food availability, fish densities may also have been influenced by water flow and temperature differences.

Fish habitat is further degraded when manure from livestock enters surface waters as runoff and increases levels of ammonia and salts. The biochemical oxygen demand created through manure inputs can also decrease the dissolved oxygen available to stream fauna. The average diary cow produces 55 kg of manure and 17 kg of urine daily, while beef cattle produce 21 kg of manure and 6 kg of urine a day (ASAE standards, 1989). If this waste is concentrated over space or time, lethal concentrations can develop.

California's population grew rapidly into the 1900s, and the increased demand for food, coupled with the abundance of fertile land in the Sacramento—San Joaquin Valley, led to opportunities for agriculture limited only by a lack of available water. The need for agricultural water and electricity for an increasing population led to the construction of numerous dams for flood control, and to provide water for agricultural diversions and hydroelectric power. In the 1930s, the Central Valley Water Project aggressively dammed rivers and diverted water to support the burgeoning agriculture industry. Dams prevented salmon from reaching hundreds of kilometers of potential spawning grounds. Fish present in rivers were often lost through unscreened electrical turbine intakes and agricultural diversions. In California's Central Valley, there are over 2000 unscreened water diversions. Salmon lost over 160 km of habitat in the upper Trinity River with construction of the Trinity Dam. The Bureau of Reclamation has at times authorized diversion of as much as 90% of Trinity River water. The current mandated Trinity flow allocation of 419 million m^3 annually are thought by many to be insufficient to restore fish populations. In the San Joaquin River, water diversions have reduced salmon runs of 70,000 fish in 1985 to less than 1000 in recent years. The effect of these water diversions has been amplified during the last 10 years of drought conditions existing in California. Agricultural development is responsible for increased erosion, and elevated water temperatures that result from loss of riparian cover and impoundment of water in reservoirs. This habitat degradation and reduced water flow has precluded salmon from using many historical spawning and nursery areas.

The San Francisco Bay estuary is dramatically influenced by agricultural land use practices in the 160,000 square kilometers that comprise the watershed for this 4700 square kilometer estuarine delta. To increase agricultural land and provide greater flood protection, many areas have been diked and leveed, draining close to 25 million hectares of wetland which had historically acted as a buffer between the San Francisco Bay estuary and the Sacramento — San Joaquin river delta. Between 50 and 80% of the freshwater inflow to San Francisco Bay has been diverted for agriculture and residential use. The potential magnitude of these impacts on fisheries is evident from the fact that nationwide, 75% of commercially caught fish and shellfish are estuarine dependent, and in the northwest, 52% of the commercial catch utilize estuarine habitat during part of their life cycle (Chambers, 1992).

Timber harvesting practices have had profound effects on salmon habitat throughout California. Loss of stream cover increases temperatures while removing sources of large woody debris that provide nutrients and habitat complexity. Past timber harvest practices created splash dams and log jams that degraded or blocked fish habitat. Loss of woodland cover increases surface water flows which carry a higher sediment load into streams (Harr and Nichols 1993). Erosion from logging roads and culverts is the major source of sediments entering forest streams (Yee and Roelefs 1980). Impacts of logging on salmonid populations vary greatly depending on the silviculture practices being used. Poor logging practices along the Noyo River in Mendocino County decreased the biomass of steelhead and coho salmon by 42 and 65% respectively, while different logging techniques in other similar streams have been associated with increased fish biomass (Burns 1972).

Commercial and sport fisheries have also contributed to the decline of the salmon resource. Legions of sport fisherman and highly efficient commercial gill net and troll fisherman harvest substantial numbers of returning salmon. Regulatory attempts to manage this mixed stock fishery have been unable to maintain small discrete stocks that are mixed with returning wild and hatchery fish from larger populations.

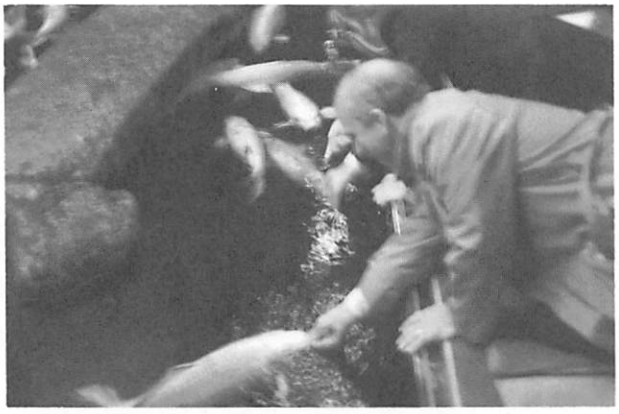
There is general recognition among fisheries managers that the crisis in anadromous fish stocks in California results from the cumulative effects of

activities in the watershed that have damaged habitat. This has led to increasing demands to improve land use practices and address critical water issues that are at the heart of the salmon decline. Federal, state, and local citizens groups are beginning to work together to solve these problems. These efforts are aimed at revitalizing salmonid populations, recognizing that restoring populations to historical levels is unlikely given the increased activities and resource utilization in the watersheds.

REVITALIZATION

Revitalizing salmon habitat to maintain viable wild stocks of salmon will require the efforts of everyone using resources within the watersheds. Good examples of activities to accomplish this can be seen in the Sacramento and San Joaquin River Central Valley Improvement Project, Endangered Species Act protection, and in management practices being developed in industry, commerce, and residential communities.

The Central Valley Project Improvement Act of 1992 (CVPIA) authorized fish and wildlife protection and dedicated 987 million m^3 of water for this purpose. In May of 1994, the U.S. Fish and Wildlife Service published the Plan of Action for the Central Valley Anadromous Fish Restoration Program to accomplish the provisions of the CVPIA. Foremost among these provisions is to develop a program that by the year 2002 will allow sustainable long-term natural production of anadromous fish in Central Valley rivers and streams at twice the average levels existing between 1968 and 1991. The most significant aspect of the CVPIA is the recognition that fish and wildlife have a legally recognized right to water. Many California rivers and streams are overallocated, and traditionally, agricultural and hydropower water rights were fulfilled prior to allocating water for fish. During periods of drought, the remaining water is often insufficient to support anadromous fish, and the last decade of drought conditions in California contributed substantially to the historically low salmon populations observed today.



While the CVPIA authorized fish and wildlife preservation and allocated water resources for that purpose, a number of Central Valley irrigation districts have filed suit in federal court to block water delivery for fish and wildlife in the San Joaquin River. It is likely that future decisions allocating water resources will increasingly be made in the courts.

In recognition of these problems and the need for scientifically valid data, steps are being taken to quantify water flow required for anadromous resources. Increased conservation and improved irrigation practices are being implemented to improve in-stream water conditions. Unscreened diversions are recognized as contributing to salmon losses and efforts are being made to install screens and modify older designs to improve their effectiveness.

The Endangered Species Act (ESA) of 1993 is powerful legislation for the protection of endangered species and their habitat. The winter run Sacramento River chinook population has been listed as endangered, and there is currently a captive breeding program underway as protection against further declines or loss of natural stocks. The spring run Sacramento River chinook population has been withheld from listing to allow local interest groups to develop a private recovery plan. This group is composed of landowners, conservationists, fishermen, and other resource users cooperating to develop a plan for fish recovery. The likelihood that the population would receive federal protection under the ESA was largely responsible for bringing traditionally competing resource users to work together to develop a recovery plan.

Timber harvest practices have been modified to substantially reduce their negative impact by reducing erosion and maintaining streamside buffer strips that provide shade, habitat, and a source of large woody debris for cover and nutrient input. The Bureau of Land Management and U.S. Forest Service have developed a "PACFISH" strategy that includes the following criteria for stream protection buffer strips (Philips 1994):

- ⇨ Fish bearing streams and lakes—300 feet (100 m)
- ⇨ Permanent non-fish bearing streams—150 feet (50 m)
- ⇨ Ponds, reservoirs, and wetlands > 1 acre—150 feet (50 m)
- ⇨ Intermittent streams, wetlands < 1 acre (0.4 hectare) and erosion prone areas—100 feet (30 m)

In an effort to improve water quality and fish habitat the Federal Government in 1972 passed the Clean Water Act designed to reduce pollution from point source discharges. Having achieved good success with this program, but recognizing the need for further

improvement, the Clean Water Act was amended in 1987 to include non-point source discharges. This legislation has aided in the focus seen in many areas on watershed management, and the cumulative impact of land use practices on fish populations. This increased awareness, coupled with conservation and better management practices, can revitalize salmon habitat in order to maintain sustainable wild anadromous fish populations.

LITERATURE CITED

ASAE, 1989. Manure production and characteristics. Engineering Practices Subcommittee of the ASAE Agricultural Sanitation and Waste Management Committee. American Society of Agricultural Engineers D384.1, pps. 437–439.

Burns, J. W. 1972. Some effects of logging and associated road construction on northern California streams. Trans. Am. Fish. Soc. 101(1): 1–17.

Chambers, J. 1992. Coastal degradation and fish population losses. In R. Stroud (ed.), *Stemming the Tide of Coastal Fish Habitat Loss*, pp. 45–51. National Coalition for Marine Conservation, Savannah, GA [258 pp].

Harr, R. D. and R. A. Nichols, 1993. Stabilizing forest roads to help restore fish habitats: a northwest Washington example. Fisheries 18: 18–22.

Nehlsen, W., Williams, J. and J. Lichatowich. 1991. Pacific Salmon at the Crossroads: Stocks at Risk from California, Oregon, Idaho, and Washington. *Fisheries* 16 (20: 4–21).

Phillips, S. 1994. Vital habitat concerns. Pacific Fisheries Management Council Habitat Committee. National Oceanic and Atmospheric Administration award # NA47FC0025. 17 pps.

Wohl, N. E. and R. F. Carline. 1995. Relations among riparian grazing, sediment loads, macroinvertebrates, and fishes in three central Pennsylvania streams. Can. J. Fish. Aquat. Sci. (submitted)

Yee, S. and T. Roelofs. 1980. Planning forest roads to protect salmon habitat. In W. R. Meehan (ed.), *Influence of Forest and Rangeland Management on Anadromous Fish Habitat in Western North America.* US Dept. Agriculture, Forest Service, Pacific Northwest Forest and Range Experiment Station, Portland, OR. (General technical report PNW; 109) Government document number A 13.88: PNW–109, 26 pp.

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MARINE RANCHING OF JAPANESE FLOUNDER BY ACOUSTIC TRAINING

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ABSTRACT

The experimental marine ranching of Japanese flounder by acoustic training has been carried out at Marino Forum 21 and Niigata Prefecture since 1991. This paper introduces the conception, methods, and results of the acoustic training. It was observed by the preliminary experiment that Japanese flounder can be trained by acoustic training. Furthermore, the experiment by using a platform pointed out that the acoustic training increased the recapture rate of the released fish.

INTRODUCTION

Japanese flounder (*Paralichithys olivaceus*) is one of the most highly valued fish in Japan. Recently, reared juveniles of Japanese flounder were released for enhancement of resources. The number of released juveniles is increasing year by year, and about 18 million juveniles were released in 1992.

Marine ranching is a system like a ranch in the sea. Namely, it is a new fishery system to breed and control coastal marine resources by means of improved growing areas, releasing reared juveniles, and preservation of environment and fisheries management. It changes the old type of capture fisheries and improves productivity.

The experimental marine ranching of Japanese flounder by acoustic training has been intensely carried out at Marino Forum 21 and Niigata Prefecture under the conception of the seabed ranching in Mano Bay of Sado Island(Anzawa 1994). This new system aims for improved survival rate after release, contribution to the expansion of production of Japanese flounder fisheries, and technological development of the controlled fisheries for resource management.

CONCEPTION

Fish fed with a sound repeatedly gather by learning, when if only the sound is made. In acoustic training this habit of fish is made use of. Trained fish stay around the acoustic feeding area after liberation into the open area without an enclosure. It is the most significant merit for acoustic training that released fish could be kept in certain areas and be well managed.

Figure 1 shows the scheme of the seabed ranching in Mano Bay. There is an acoustic training platform in the inner part of the bay. After acoustic training the juveniles remain around the platform for a time. Then, they move gradually into areas for growing in the central part of the bay where artificial fish banks were sunk. After growing in this area, adult fish are caught in the fishing ground at the bay entrance.

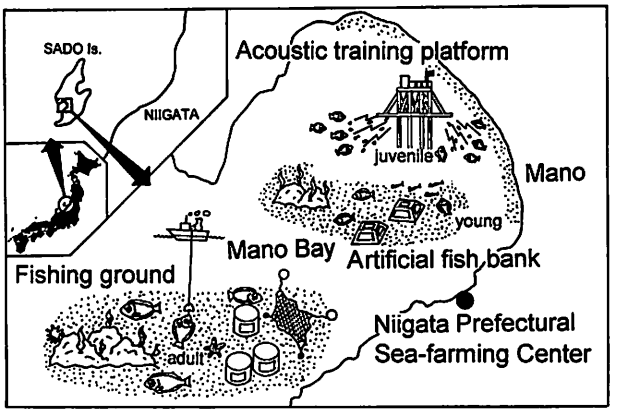


Figure 1. The concept of "seabed ranching in Mano Bay"(modified from Anzawa 1994).

PRELIMINARY EXPERIMENT

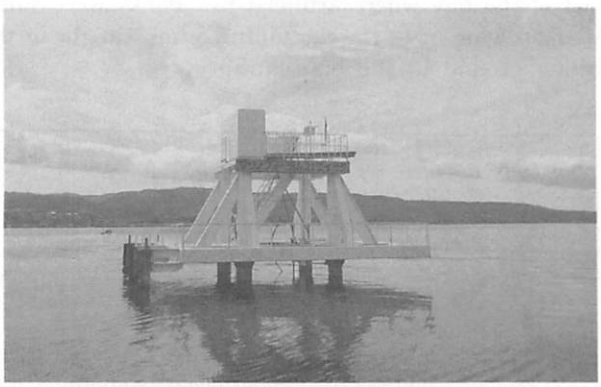
The preliminary experiment of the acoustic training was carried out in overland concrete tanks. Juvenile Japanese flounder, 40 and 90 mm in total length were examined. Artificial pellets were fed 3 times per day in

acoustic training. The results are as follows: (1) More than 90% of fish made a response to sound 10 days after the beginning of acoustic training; (2) About 80% of the trained fish made a response to sound after 10 days interruption of the acoustic training, and about 50% of the fish reacted after 20 days interruption; (3) The smallest sensitive sound pressure was about 100 dB. The highest endurable sound pressure was more than 162 dB. Thus, it was observed by the preliminary experiment that Japanese flounder can be trained by acoustic training.

EXPERIMENT WITH THE PLATFORM

DESIGN AND MATERIALS

An experimental acoustic training platform was constructed at 500 m offshore, 5-m depth in the inner part of Mano Bay of Sado Island, Niigata Prefecture in 1991 (Figure 1). The platform is made of four driving piles in order to resist strong waves, and it has two floors (Figure 2). The lower floor is 10 m^2 and 1.5 m above the sea surface to hang the enclosure net (Figure 3). An underwater speaker is placed 50 cm under the sea surface. A fish finder is set to observe the reaction of fish against sound. There are many kinds of equipments for measurement, control of sound and feeding, telegraph, and solar power supply on the upper floor, which is 5 m above the sea surface. The telegraph system can control the sound and feeding, and confirm the record of the fish finder from a land bureau at Niigata Prefectural Sea-farming Center.



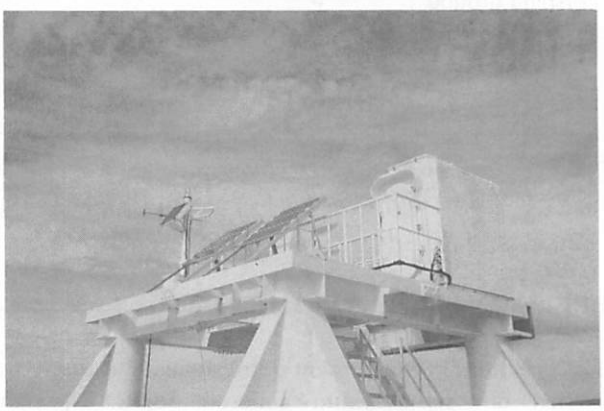


Figure 2. Photographs of the acoustic training platform. A: whole, B: upper floor.

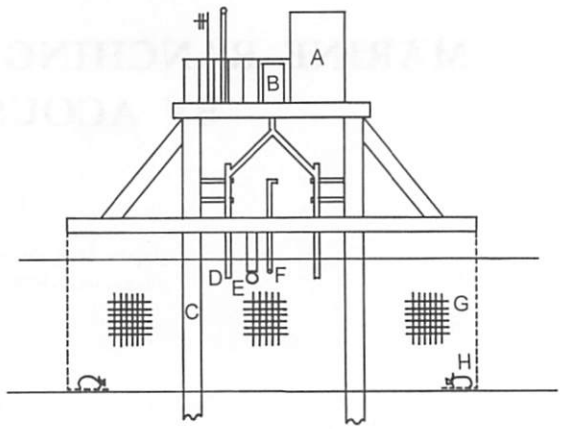


Figure 3. A design of the acoustic training platform (modified from Anzawa 1994). A: control system and power supply, B: feeding machine, C: piles, D: pipe for feeding, E: underwater speaker, F: transducer of fish finder, G: enclosure net, H: sand bag.

Japanese flounder were reared with acoustic training by using this platform. After 43,000 Japanese flounder of 43-mm average total length with Alizarin complexion marking were put into a 10-m angular enclosure net on 2 August 1991, acoustic training was started. Artificial pellets were fed 6 times per day with 150-dB intermittent sound of the sine wave of 300 Hz. The enclosure net was removed on 11 September, 40 days after starting.

The investigations and the measurements at fish markets were carried out. The catch of Japanese flounder in Mano Bay, the rate of released fish in the catch, and the number of the acoustic trained fish were investigated. And the recapture rate of the released acoustic trained fish was calculated.

RESULTS AND DISCUSSION

Figure 4 shows the records of the fish finder that caught the fish. Remarkable rising of the fish school from the seabed, which is thought to be the reaction against sound, was recognized with in 6 days after starting. Then, the reaction against sound increased day by day, and the rising of the fish school became more than 3 m above the seabed with in 16 days after starting.

After removing the enclosure net, Japanese flounder feeding on the pellets were observed. However, the seabed around the platform was washed and dug by a typhoon on 28 September 1991, 17 days after removing the enclosure net, and the acoustic training fish

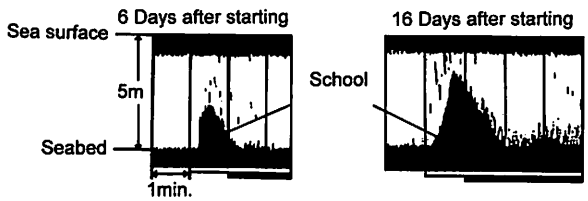


Figure 4. Records of the fish finder (modified from Anzawa 1994). Upper lines are the sea surface, and bottom lines are the seabed. White bars and black bars show the period of sound and the period of feeding, respectively. Vertical lines show 1 minute intervals.

dispersed.

The estimated number of fish when the enclosure net was removed, was 16,500. Survival rate in the enclosure net was 39% and it was a low value in comparison with the average in the overland tank. The average total length when the enclosure net was removed, was 113 mm. The growth rate was 1.9 mm a day. It was 20% to 30% better than that in the overland tank.

The number of the acoustic trained fish released in Mano Bay in 1991 was about 16,500, and their estimated catch was 3,544 by the end of 1993. Therefore, the recapture rate of the released acoustic trained fish is about 22% at 2 years and 4 months, and it is the highest value of the recapture rates of the Japanese flounder released in Mano Bay until now.

One primary factor of improvement of the recapture rate of the released acoustic trained fish in this experiment is probably that the fish were getting adapted to the natural environment by the acoustic training. In fact, Furuta (1993) reported that the juveniles which were reared under a nature-like environment improved their ability to avoid predation. The body size of the juveniles is also an important factor. The total length of the Japanese flounder released in Mano Bay without acoustic training was about 40 mm. On the other hand, that of the acoustic trained fish was 113 mm. It is known that large juveniles are recaptured more than small ones. It is concluded that these factors improved the recapture rate of the released fish. Therefore, marine ranching with acoustic training should be one direction of future fisheries.

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LITERATURE CITED

Anzawa, W. 1994. Research and development of the creating techniques for Marine ranching of Japanese flounder, Marinoforum 21 Niigata. In: MF21 No. 32, pp. 90–96. Tokyo, Marino Forum 21. (In Japanese).

Furuta, S. 1993. Releasing techniques and fry quality. In: Healthy Fry for Release, and Their Production Techniques, Edited by Chikara Kitajima, pp. 94–101. Tokyo, Kouseisya-Kouseikaku. (In Japanese).