# Marine Farming and Enhancement

Proceedings of the Fifteenth U.S.-Japan Meeting on Aquaculture Kyoto, Japan October 22-23, 1986

Albert K. Sparks (editor)

U.S. Department of Commerce

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# NOAA Technical Report NMFS 85

# Marine Farming and Enhancement

Proceedings of the Fifteenth U.S.-Japan Meeting on Aquaculture, Kyoto, Japan October 22-23, 1986

Albert K. Sparks (editor)

Panel Chairmen: Conrad Mahnken, United States Ikuo Ikeda, Japan

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

March 1990



U.S. DEPARTMENT OF COMMERCE Robert Mosbacher, Secretary National Oceanic and Atmospheric Administration John A. Knauss, Under Secretary for Oceans and Atmosphere National Marine Fisheries Service William W. Fox, Jr., Assistant Administrator for Fisheries

## 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 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 was begun during the Third Cabinet-Level Meeting of the Joint United States-Japan Committee on Trade and Economic Affairs in January 1964. In addition to 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.

Conrad Mahnken - United States Ikuo Ikeda - Japan

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# A New Internal Telemetry Tag for Fish and Crustaceans

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### ABSTRACT

An ongoing cooperative agreement between the Bonneville Power Administration and the National Marine Fisheries Service was initiated in 1983 to evaluate the technical and biological feasibility of adapting a new identification system to salmonids. The system is based on a passive integrated transponder (PIT) tag. Each tag measures 12 mm in length by 2.1 mm in diameter and is uniquely coded with one of 34 billion codes. The tag's operational life is unknown at this time; however, it is thought to be 10 or more years. The tag can be detected and decoded in place, eliminating the need to anesthetize, handle, or restrain fish during data retrieval.

Biological tests indicate the body cavity of juvenile and adult salmonids is biologically acceptable for tag implantation. Comparisons between PIT-tagged and traditionally tagged and marked juvenile salmonids are discussed. Laboratory and field tests showed that the PIT tag did not adversely affect growth or survival, nor was there any appreciable tissue response to the tag. No evidence of infection due to tagging procedures was observed. Video-taped swim-chamber tests showed no significant effect of the PIT tag on respiratory rate, tail beat frequency, stamina, or post-fatigue survival of juvenile salmonids. Tag retention within the body cavity was near 100% for salmonids weighing from 2 to 10,000 g. Previously PIT-tagged mature salmon which were hand stripped of sperm and eggs showed high tag retention with no adverse tag-caused effects.

During their outmigration, PIT-tagged juvenile salmonids were successfully interrogated at two dams using automatic tagmonitoring equipment. All data were automatically recorded and stored by computer. PIT-tag reading efficiency was 96 to 100%, while reading accuracy was over 99%. The tag-monitoring equipment proved to be reliable under field conditions.

Special tagging considerations with Crustacea and preliminary testing of the PIT tag with two crustacean species are discussed, along with future applications of the PIT tag to fisheries research. The recognition of an animal or a group of animals within a population is important for many reasons in fisheries research. Many types of tags and marks have been developed to aid biologists in recognizing animals (Rounsefell 1963, Farmer 1981). Unfortunately, no one technique has been totally satisfactory from a biological or technical standpoint. In 1983, the National Marine Fisheries Service began a study supported by the Bonneville Power Administration to evaluate the technical and biological feasibility of adapting a new identification system to salmonids. The system is based upon a passive integrated transponder (PIT) tag. This tag has the promise of eliminating some of the inherent problems with present tagging and marking systems. In addition to the research with salmonids, preliminary tagging studies have also been conducted with two crustacean species. This paper provides an overview of the basic tag operation, biological acceptability in test animals, field testing results, and a discussion of some of the possible applications of the PIT tag.

## Tag operation \_\_\_\_\_

The PIT tag consists of an antenna coil that has about 1,500 wraps of a special coated, 0.0254-mm diameter copper wire. The antenna coil is bonded to a integrated circuit chip. The electronic components of the tag are encapsulated in a glass tube about 12 mm long and 2.1 mm in diameter (Fig. 1). Each tag is preprogrammed at the factory with one of about 34 billion unique code combinations. The tag is passive, having no power of its own, and thus must rely upon an external source of energy to operate. A 400-KHz signal energizes the tag, and a unique 40-50 KHz signal is transmitted back to the interrogation equipment where the code is immediately processed and displayed, transmitted to a computer via an RS-232 interface, and/or placed on printed hard copy. A portable hand reader (Fig. 2) or a fixed tag-monitor system is used to interrogate and display the tag code information. Data transfer rate is 4,000 bits/s. The interrogation range of the tag varies with the monitoring equipment used: Using a hand reader the reading range is up to 7.6 cm, while with a fixed full-loop interrogator the reading range of detection is about 18 cm (Fig. 3). The tag can be read through



Figure 1 PIT tag.



Figure 2 Portable hand-operated PIT-tag reader.



Figure 3 Typical PIT-tag monitoring system for dams.

soft and hard tissue, liquid (seawater and freshwater), glass, and plastic, but not through metal. Extreme heat or cold (60 to  $-90^{\circ}$ C) does not appreciably affect detection or reading of the tag. Successful tag monitoring can take place at velocities up to 30 cm/s.



Figure 4 Comparison of length change between PIT-tagged (broken line) and control (solid line) fall chinook salmon (1984 brood) over time.

No special permits are required of the operator other than those obtained from the Federal Communications Commission (FCC) or their equivalent for the operation of lowpowered transmitting devices. These permits pertain only to specialized monitoring systems and not the hand-held system already certified by the FCC. No special training or licensing of the operator is required to operate the tag-monitoring equipment.

PIT tag operational life is currently being investigated. Two 300-fish test groups of juvenile fall chinook salmon were established: One control group (no tag), and one tag group. All fish in each test group were weighed and measured at the time the test groups were established. The two test groups were maintained in freshwater until smolted and then transferred to seawater where they are being held in separate sea cages. Observations on growth, survival, and tag retention and operation were made at various intervals. Results after 250 days show no meaningful difference in growth (Fig. 4) or survival between groups of tagged and control fish. Tag retention and operation have been 100%. Because of the passive nature of the tag, an operational life of 10 years or more is expected.

# **Biological suitability: Salmonids** -

It is important that a tagging system does not alter growth, survival, behavior, or reproduction. In addition, tag longevity (tag retention and operational life) is an important consideration. Laboratory tests were conducted to examine these factors as they apply to the use of the PIT tag with salmonids. Juvenile and adult chinook (*Oncorhynchus tshawytscha*), Atlantic salmon (*Salmo salar*), and steelhead (*Salmo gairdneri*) were used in the studies. The fish ranged in weight from 2 to 10,000 g. All tags were injected into the body cavity using a modified hypodermic syringe and a 12-gauge needle (Prentice et al. 1986).

## **Tissue response**

Adverse tissue response to the tagging needle and tag has been minimal. Tag-wound condition and tag placement within the body cavity were documented by sacrificing groups of juvenile fall chinook salmon over time (Table 1). In nearly 85% of the fish examined (n = 195) the tag wound was completely healed by day 40-45, with only a scar indicating the area of needle insertion. At the end of this same period, 7.3% of the fish had an open wound and 8.3% had a wound that was closed but slightly discolored. All fish (n = 99) sacraficed 97 days post-tagging showed complete healing of epidermal and subcutaneous tissue. A the termination of the study (day 127) an additional 102 fish were sacrificed; 99.2% had completely healed tagging wounds, 0.6% had open wounds, and 0.2% had wounds that were closed but discolored. The study also indicated that once the tag was injected into the body cavity, its location was stable over time. The majority of tags were found near the posterior end of the pyloric caeca.

## Effects of maturing fish

Numerous morphological and physiological changes take place as salmon mature. These changes may alter the response of fish to foreign material such as a PIT tag. Furthermore, it is necessary to know whether a tag placed in the body cavity would cause internal damage to eggs and whether a tag would be retained during spawning. A study addressing these issues was conducted using 21 male and 60 female maturing Atlantic salmon. The fish ranged in weight from 2,500 to 10,000 g and in length from 61 to 80 cm. All fish were PIT tagged intraperitoneally using the method of Prentice et al. (1986). The fish were examined several times prior to spawning to determine wound condition, tag retention, readiness to spawn, and general condition, and scanned for tag code using a hand-held scanning unit. When fish were determined to be ready to spawn, eggs were collected by hand stripping. Individuals that spawned were subject to 1-4 strippings.

During the study, no adverse tissue reaction was noted. All tagging wounds were closed and healing by the third day after tagging. No infection or discoloration was noted in the area of the tag. All 21 males matured, and milt was collected

#### Table 1

Summary of wound condition after tagging and tag location within the body cavity of juvenile fall chinook salmon over time with descriptions of wound condition and tag location codes.

	Days post-tagging						
Code	40-45	97	127				
	Percent fish within a classification c						
Wound code <sup>1</sup>							
Α	7.3	0	0.6				
В	8.3	0	0.2				
С	84.4	100.0	99.2				
Tag location code <sup>2</sup>							
A	2.1	0	3.9				
В	86.5	69.1	83.3				
С	0.0	4.4	1.0				
D	5.2	25.0	6.9				
E	6.3	1.5	4.9				

<sup>1</sup>A Open wound.

- B Wound that is closed by a thin membrane and is healing; at times a slight red or pinkish coloration is noticeable in the area of the wound.
- C Wound completely healed that may or may not be noticeable by the presence of a scar. No red or pink coloration in the area of the wound.
- <sup>2</sup>A Tag located between pyloric caeca and mid-gut.
- B Tag located near abdominal musculature and often embedded in the posterior area of pyloric caeca near the spleen or in adipose tissue at the posterior area of pyloric caeca.
- C Tag found in an area other than those noted; generally between mid-gut and air bladder or between liver and pyloric caeca.
- D No tag present.
- E Tag partially protruding through abdominal wall.

from each fish. Tag retention was 100% for the males. A total of 48 females were spawned. Tag retention was 83% for spawning females and 100% for non-spawners. Four tags were passed during the first stripping and four tags during the second-fourth stripping (Table 2). When a tag was passed, it was easily recognized among the eggs. The presence of tags caused no observable adverse effects on the eggs.

Table 2           Spawning dates and PIT-tag rejection by female Atlantic salmon							
Date spawned	No. females spawned	Cumulative no. spawned	No. tags not retained				
21 Oct	21	21	1ª				
22 Oct	4	25	0				
23 Oct	7	32	0				
25 Oct	7	39	2 <sup>b</sup>				
29 Oct	3	42	3°				
4 Nov	6	48	2 <sup>d</sup>				

<sup>c</sup>One tag not retained during 1st, 2d, and 4th stripping.

<sup>d</sup>Two tags not retained during 1st stripping.

Treatment* and		Test length	Size	Size (g)		DIT to a notantion
test group	No. days	(g)	start	end	Survival (%)	PIT-tag retentior (%)
Control-well	202	135	4.9	24.9	100.0	
Control-stream	200	135	5.1	24.8	99.0	_
PIT tagged						
well #1	201	139	3.2	20.5	99.5	100.0
well #2	200	135	5.1	27.4	100.0	100.0
well #3	201	134	7.1	25.9	100.0	100.0
well #4	200	137	9.7	32.6	97.0	100.0
stream #1	200	139	3.2	21.1	95.0	99.0
stream #2	200	135	4.8	22.6	98.0	100.0
stream #3	203	134	7.3	29.9	95.0	100.0
stream #4	202	137	10.0	30.3	98.0	100.0

# Growth and survival

Tests were conducted in 1986 using juvenile fall chinook salmon to determine the minimum size that could be successfully PIT tagged. Fish were tagged at four size ranges and held in separate holding containers (Table 3). The number of fish in each test group ranged from 200 to 203. Fish ranged in weight and length from 1.7 to 14.9 g and 56 and 120 mm, respectively, at the time of tagging. Two separate water supplies (well water and stream water) were used in the study to determine if exposure to water containing fish pathogens might affect tag-wound healing or tag retention. Four sets of weight and length data were obtained on each group of fish during a 134-139 day period. Tag retention was excellent for both groups (99-100%). Growth comparisons (both between the PIT-tagged well- and stream-water groups, and with the control groups) indicated slight differences in length and weight at some sampling periods. However, there appears to be no observable pattern to the differences, suggesting that the glass-encapsulated PIT tag does not compromise growth in juvenile salmonids reared in either well- or streamwater. Range of overall (134-139 days) survival of PIT-tagged fish was 97-100% in the well-water groups and 95-98% in the stream-water groups. Visual inspection of the data (Table 3) shows that mortality occurred in the smallest size groups of fish for both well- and streamwater groups. Examination of mortalities for both initial welland stream-water groups showed perforation of the intestine as the cause of death. Four of the seven mortalities in the first stream-water test group occurred within the first 2 days after tagging and were from the first 10 fish tagged. Because this was the first group of fish to be tagged in the year, our tagging technique was not up to standard. Tagging technique was refined and no further problems with intestinal perforation was observed in the other test groups. Mortality in the larger size groups was variable (5% or less) and occurred

primarily in the stream-water held groups (Table 3). Visual examination indicated that these populations of fish were in various stages of smoltification. Reductions in immune response have been noted during smoltification (Maule and Schreck 1987). It is possible that exposure to pathogens in the stream water, and/or smoltification status itself, contributed to these mortalities. The data suggest that fish weighing 3 g (mean weight) or less, or those undergoing smoltification, experience a low mortality (5% or less) when PIT tagged.

# Effects on swimming ability

Tests were conducted to evaluate the physiological/behavioral effects of the PIT tag on swimming ability in juvenile steelhead. The test were conducted in a modified version of a Blaska respirometer-stamina chamber described by Smith and Newcomb (1970) (Fig. 5). Two size ranges of fish were tested. The first group, tested in July 1985, averaged 81 mm in length and 6.5 g in weight. The second test group, in October 1985, averaged 112 mm in length and 17.2 g in weight. In both tests a random sample of fish (n = 200) was removed from the main population and intraperitoneally tagged with PIT tags using the procedures of Prentice et al. (1986). A control (non-tagged) group (n = 200) was also established from the main population at this time. Swimming tests were conducted on days 0 (same day as tagging), 1, 2, 3, 4, 7, 9, 11, 14, 17, 21, and 25, with 12 tagged and 4 control fish tested each day. All tests were recorded on video tape and monitored at slow speed to determine swimming stamina (time to impingement), tail-beat frequency per minute, respiratory rate (opercular rate/min), and stride efficiency (no. tail beats/min required to maintain a unit swimming speed of one body length/s). All tested fish (tagged and control) were held for 14 days post-test to establish stress survival profiles.



Figure 5 Blaska respirometer-stamina chamber.

The swimming stamina, stride efficiency, and respiratory rate data were compared between tagged and control fish, and between post-tag testing data using the non-parametric Mann-Whitney test. All data analyses followed the methods of Sokal and Rohlf (1981). The data indicated that neither the act of tagging nor the presence of the PIT tag compromised swimming stamina, stride efficiency, or respiratory rate of juvenile steelhead. In addition, post-test survival was not affected by the PIT tag, and tag retention was 100%. At the termination of the post-test holding period, all PITtagged fish were sacrificed and necropsies performed to determine tissue reaction to the tags. No adverse tissue reactions or tag migrations within the peritoneal cavity were noted.

# Comparisons with traditional tagging and marking methods

A series of tests comparing the PIT tag to traditional methods of marking and tagging was conducted under field conditions using active, outmigrating spring chinook salmon, fall chinook salmon, and steelhead. The tests were conducted at Lower Granite Dam on the Snake River and McNary Dam on the Columbia River. The survival of PIT-tagged fish was compared with that of control fish (handled but not tagged), coded-wire tagged (CWT), CWT plus cold branded, and cold branded. Fish from all treatments were combined in a common holding cage, since each treatment could be recognized by its identifying mark or tag. Five replicates of 25 fish per treatment for a total of 125 fish per replicates were used in the 1985 test. In the 1986 tests, 20 fish per treatment were used for a total of 100 fish per replicate. The fish were held for 14 days in five cages that received a continuous supply of untreated ambient river water. The fish were examined daily for mortality.

No difference in survival between fish injected with the PIT tag and in the other treatment groups was noted at the end of 14 days of holding (Table 4). Mortality varied between dams but not between test groups at a dam. All PITtagged fish showed complete closure of the tagging wound at the end of 14 days. No infection or fungus was observed around the tagging would prior to healing.

Table 4           Summary of tests comparing the survival of PIT-tagged fish with that of traditionally tagged and marked fish at dams along the Snake and Columbia rivers.									
		t.			Survival (%)	)			
Location	Species	Days observed	Control	PIT	Cold-branded	CWT	CWT + cold-branded		
Lower Granite (1986)	Spring chinook	14	95	98	96	97	99		
Lower Granite (1986)	Steelhead	14	100	99	100	99	97		
McNary (1986)	Spring chinook	14	86	83	86	80	89		
McNary (1986)	Steelhead	14	89	87	93	91	94		
McNary (1986)	Fall chinook	14	64	65	59	68	66		
McNary (1985)	Fall chinook	14	96	87	94	92	93		



Figure 6 Location of hydroelectric dams on the Snake and Columbia rivers.



Figure 7 Typical hydroelectric dam with juvenile salmon collection facilities.



Figure 8 Location of PIT-tag monitors at Lower Granite Dam, Snake River.

# Tag detection at dams

Outmigrating salmonids on the Columbia River system are confronted with a number of hydroelectric dams that cause decreased migration rates and increased mortality (Fig. 6). Several of these dams have been modified to collect and/or divert migrants around them as a method of increasing overall survival in the system. The collection facility generally consists of a series of traveling screens that divert fish from the dam's turbine intakes and eventually into a gallery of pipes that lead to a wet separator (Fig. 7). The separator reduces the volume of water carrying the fish and removes debris. Fish are then diverted either to a raceway for later transport downstream via truck or barge, or directly to a barge for transportation downstream, or back into the river. A subsample of the fish exiting the wet separator is diverted into a holding tank and then to an observation room where they are examined for tags and marks.

Traditionally, methods such as branding and coded-wire tagging (CWT) have been used to evaluate outmigration suc-



Figure 9 Location of PIT-tag monitors at McNary Dam, Columbia River.

cess. However, because of the unique features of the PIT tag, it could be used in place of the traditional methods, generating better results statistically while using significantly fewer fish. With this goal in mind, prototype PIT-tag monitoring systems were installed at two dams. The monitors were located at the juvenile fish collection facilities at Lower Granite Dam on the Snake River and McNary Dam on the Columbia River. The monitors were placed in positions insuring that 100% of the fish exiting the wet separator were monitored (Figs. 8, 9).

A series of tests was conducted to evaluate the operational reliability, tag reading accuracy (correct decoding of the tag), and reading efficiency (percent tagged fish detected) of the dam PIT-tag monitors. Migrating juvenile spring chinook salmon, fall chinook salmon, and steelhead were used as experimental animals. The tests consisted of releasing 480 PIT-tagged fish in front of the tag monitors. Tag detection efficiency ranged from 96 to 100%, while tag reading accuracy was over 99%. The monitoring equipment remained in an active state at the dams for up to 7 months without major problems. The PIT-tag monitoring system proved to be reliable, efficient, and accurate under field conditions.

Table 5           Summary of data obtained from the release of PIT-tagged and cold-branded fish into McNary Dam Reservoir, Columbia River, 1985 and 1986.								
Year	Species	Treatment	Total fish tagged and branded	Pre-release mortality (%)	Total fish handled	No. fish observed	Percent observed	SD (%)
1985	Fall chinook	Branded	4,000	2.3	13,239	53	19.4*	9
1985	Fall chinook	PIT tag	400	1.5	400	64	16.2	4
1986	Fall chinook	Branded	5,000	3.8	201,670	95	27.4*	4
1986	Fall chinook	PIT tag	500	3.6	500	142	28.4	1
1986	Spring chinook	Branded	5,000	1.5	154,826	194	38.9*	10
1986	Spring chinook	PIT tag	500	1.0	500	318	63.6	2

## Table 6

Summary of data obtained from the release of PIT-tagged and cold-branded fish from Dworshak National Fish Hatchery, Snake River, 1986.

					Monitor location						
				Pre-release	Lower Granite Dam			McNary Dam			
Species Treatm	Treatment		Total released	Fotal mortality	No. fish observed	Expanded*	Percent observed	No. fish observed	Expanded*	Percent observed	
Spring chinook	Branded	41,584	40,675	2.2	474	4,659	11.5	362	3,402	8.9	
Spring chinook	PIT tagged	2,500	2,450	2.0	464	_	18.9	264		10.8	
Steelhead	Branded	35,372	35,025	1.0	571	7,061	20.2	39	389	1.1	
Steelhead	PIT tagged	2,466	2,424	1.7	928	_	38.1	45	—	1.8	
*No. fish observ	ed multiplied b	by a factor to	correct for	subsampling a	t the dam.						

Additional tests comparing branded and PIT-tagged juvenile migrants (fall chinook salmon, spring chinook salmon, and steelhead) were made in the field. The fish were released into the Snake River of McNary Dam Reservoir and monitored as they passed through either Lower Granite Dam or McNary Dam juvenile collection and monitoring facilities. In order to obtain sufficiently accurate information on the branded fish, large random subsamples of migrating juveniles, some of which were branded, were diverted into collection chambers. The subsampled fish were anesthetized and examined visually for brands. On the other hand, PIT-tagged fish were automatically interrogated as they passed by a dam equipped with a PIT-tag monitor system. As each PIT-tagged fish was detected, the tag information, time, date, and location of the fish was automatically entered into a computer and printer. Tables 5 and 6 summarize the results of these tests. Because branded fish were subsampled, they were detected at a much lower rate than PIT-tagged groups. An expansion factor was applied to the brand information to obtain an estimation of the true number of branded fish collected (expanded observation value). Since the retrieval of PIT-tag information is based on the monitoring of 100% of the fish passing the collection facility at a dam, no expansion factor is required and 90-95% fewer PIT-tagged fish are needed for a study. Pre-release mortality in the branded

and PIT-tagged fish was similar for each test. Use of the PIT tag also allowed the handling of substantially fewer fish than did the branding technique to obtain statistically similar results. Fish in the brand treatment were handled at the time they were branded and again while being examined at the collection facility, along with many nonbranded fish. PIT-tagged fish, on the other hand, were handled only at the time of tagging. It is concluded that the PIT-tagged fish were not compromised by the tag when released into a river or reservoir and that the PIT tag offers substantial gains in efficiency over branding for many applications.

# PIT tagging of crustaceans \_\_\_\_

Permanent identification using external tags and marks for Crustacea has been difficult because of frequent molting. External tags and marks are often lost at the time of molting or can interfere with the molting process, thus altering the animal's behavior or physical well-being. Internal coded wire (CWT) tags can eliminate the problem of tag loss at molting but require the host to be sacrificed to retrieve the tag information (Prentice and Rensel 1977). The new PIT tag has the potential to eliminate these problems. Preliminary experiments using the PIT tag with two species of Crustacea, *Macrobrachium rosenbergii* and *Cancer magister*, have been conducted. The prawns (n = 58) ranged in carapace length from 11 to 41 mm and in weight from 1.5 to 45.3 g. The crabs (n = 52) ranged in width from 64 to 130 mm and in weight from 44.4 to 273.2 g. All crabs were tagged in the thoracic sinus (hemocoel) while the prawns were tagged in either the thoracic sinus or abdominal musculature. Results for both species showed that the tag was retained through molting and the tag information could be obtained rapidly without sacrificing the tagged animal.

# Future applications \_

Based upon biological and technical information gathered to date and its unique characteristics, the PIT tag will become a valuable tool for a variety of applications in the laboratory and field. Its use will not be limited to salmon, prawns, and crabs but will be applicable to any animal that can accept and retain the tag without compromise. Examples of advantages and applications of the PIT tag include: (1) Individual identification of broodstock; (2) use with groups of animals where serial measurements, e.g., growth, of individual animals are required without sacrificing the animal; (3) reduction in the number of replicated treatments in a study because each animal is uniquely numbered and can be treated as a replicate; and (4) the ability to physically combine different treatments, since individual animals can be identified, removing the variable of rearing-container effect. Other applications might include use in behavioral studies where the movement of animals can be monitored automatically or through capture-recapture methods. It is conceivable that one could monitor bottom-dwelling PIT-tagged individuals through a grid monitor or a monitor system mounted to an underwater sled.

The main limitation to the use of the PIT tag, other than cost and physical and operational constraints, lies, as with most tools, in our imagination. The PIT tag is only the first generation of a number of sophisticated identification systems growing out of our computer age. We must utilize the full potential of these new tools if we are to meet the many challenges of fisheries enhancement and aquaculture.

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# **Preliminary Results of Red Drum Stocking in Texas**

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## ABSTRACT

The ability to control spawning and to rear red drum (Sciaenops ocellatus) in captivity has afforded managers the opportunity to use stocking to enhance native fisheries. This paper presents preliminary results of the effects of 2 years of intensive stocking in two Texas estuaries. Catch rates in gill nets fished randomly in stocked and unstocked bays in spring (April-June) and fall (September-November) were compared to determine changes in relative abundance of fishable populations. Landings by private sport-boat anglers in each bay during the lowuse (mid-November through mid-May) and high-use (mid-May through mid-November) seasons before and after stocking were compared for fish ≥450 mm total length. Relative abundance and angler landings of red drum were higher after stocking in the stocked bay; abundance and angler landings were similar or lower in unstocked bays after the stocking dates. Additional research is needed to determine optimum stocking rates, times, and fish sizes.

Red drum (Sciaenops ocellatus) is a quasicatadromous sciaenid that ranges from Tuxpan, Mexico, in the Gulf of Mexico to Massachusetts in the Atlantic Ocean (Matlock 1984). Adults spawn in oceanic waters nearshore, and larvae are carried by currents through passes into estuarine nursery areas where they remain for 3 to 5 years before returning to the ocean to spawn. Economically important sport and commercial artisinal fisheries have existed since the 1800s in Texas, Louisiana, and Florida (Matlock 1980). However, harvest is being increasingly restricted to insure adequate reproduction and growth to maintain the fishery. No red drum caught in Florida or the U.S. Fishery Conservation Zone in the Gulf may be retained, and fish caught in Texas and Alabama may not be sold. Size, bag, and possession limits exist in all five Gulf states. For example, only five red drum, all between 457 and 762 mm TL (total length), can be retained per day in Texas. However, harvest regulations are not the only options available to managers.

Stocking red drum reared in captivity has recently become another management tool for this fishery (Rutledge and Matlock 1986). In 1974 red drum were first spawned and reared in captivity (Arnold et al. 1977). The spawninginducement technique, i.e., temperature and photoperiod manipulation, was refined so that fry could be obtained at any time (Colura et al. 1976). With fry readily available, fingerlings could be produced in ponds throughout the year, and the potential for improving the red drum fishery through stocking could be examined.

Enhancement of wild populations through stocking appeared feasible. Historic bag seine and trammel net collections indicated the habitat could support more red drum than were present in the 1970s (Matlock 1984). A preliminary evaluation of a limited stocking indicated that stocked fish survived, grew, and supplemented the juvenile population in the stocked bay (Matlock et al. 1986). However, too few fish were stocked to detect any impact on recruitment to the fishery.

These initial results were sufficient to convince sport fishermen, a major electric utility company, and the Texas Legislature that a red drum hatchery could be beneficial. The Gulf Coast Conservation Association donated \$1.4 million to build the facility (Rutledge and Matlock 1986), Central Power and Light Company provided the land, and the Texas Legislature appropriated about \$160,000 operating expenses for staff and equipment. The hatchery was designed to produce about 10 million fingerlings annually from 8 ha of earthen ponds. In each of the first 3 years of operation (1983-85), 7 to 9 million fingerlings were produced (McCarty et al. 1985, Matlock 1986). However, the impact of these stockings on sport angler landings has not been determined.

The objective of this paper is to present a preliminary assessment of the success of 2 years of stocking red drum fingerlings into two Texas bays.

# Materials and methods .

Red drum fingerlings (11-83 mm TL) were spawned and reared in earthen ponds (0.8 ha) at the John Wilson Marine Fish Hatchery (McCarty et al. 1985). Fish were transported to stocking sites (Fig. 1) in trailers fitted with a three-chamber tank ( $3.0 \times 1.2 \times 0.8$  m) that was supplied with compressed oxygen to maintain 4-10 ppm (Hammerschmidt and Saul 1985). Each chamber held about 1,950 L of water and contained 10 ppm furacin.

Fish were either stocked directly into the bay or transferred through a plastic pipe to tanks on a barge by gravity flow and then transported to stocking sites. Fish were acclimated to ambient water temperature and salinity  $(\pm 2^{\circ}C \text{ and } 5 \text{ ppt})$  at each site by exchanging water in the tanks at a rate of about 2,600 L/h. Release mortality was estimated for each load by holding 25 fish in each of 3 or 4 cages for 24 hours at the release site (Hammerschmidt and Saul 1985).

About 14 million fish were released in 1983, late 1984, and early 1985 into the San Antonio and Corpus Christi Bay systems (Fig. 1). The San Antonio Bay system received 2.3 million fish in May 1983 and 6.0 million fish in May and July 1984 (Matlock 1986). The Corpus Christi Bay system received 4.7 million fish in September and November 1983 and 250,000 in January 1985. The objective of these releases was to increase significantly (P = 0.10) the number of red drum landed by sport-boat anglers over the historic harvest in these two bay systems. These two bays were selected because they have diverse habitats and fishing pressures, no netting is allowed, the ratio of surface area to number of anglers was among the lowest on the coast, and the historic landing rates for red drum were among the highest on the coast. These characteristics should have maximized the probability of determining if the objective was met. They would also allow the inference that if stocking was effective in these bays, then it should be effective in all other bays where historic landing rates were less.

The effect of stocking was measured in four ways. Cage studies (described previously) were used to determine initial survival after stocking. Bag seines were pulled at stocking sites for  $\leq 3$  months after stocking to determine longer-term survival (>0%). Ongoing surveys of sport-boat anglers (Osburn and Ferguson 1986) and fishery-independent monitoring with gill nets (7.6, 10.2, 12.7, and 15.2 cm stretched meshes) in the two stocked bays and one unstocked bay (Crowe et al. 1986) were used to measure changes in anglers' landings and red drum relative abundance. Trends in mean landing rates in stocked bays were compared with the unstocked bay.



Figure 1 Bay systems of Texas coast. Red drum were stocked in the San Antonio and Corpus Christi Bay systems.

# Results and discussion .

Stocked fish survived the stocking process very well. The mean  $(\pm 1 \text{ SE})$  24-hour survival of fish held in cages was  $89.4 \pm 2.7\%$  in 1983 and  $86.2 \pm 2.2\%$  in 1984 (Hammerschmidt and Saul 1985, Hammerschmidt 1986). Juvenile fish were recaptured in bag seines in both stocked bay systems for up to 1.5 months after stocking (Dailey and McEachron 1986). Thereafter, fish were large enough to escape bag seines (Matlock 1984).

Stocking appears to have increased the number of red drum available for harvest in Texas bays. However, this impact was not consistent among all stockings because of a major fish kill caused by record-breaking freezing temperatures during late December 1983 and early January 1984 (McEachron et al. 1984). Over 90,000 red drum were killed coastwide, and most of the dead fish were found in the San Antonio Bay system. The mean catch rates in gill nets in the stocked Corpus Christi Bay system were much higher in the 2 years after stocking than in the years before stocking (Fig. 2). Mean catch rates in the upper Laguna Madre (control) were also higher after stocking, but were not as great as in the stocked bay. The increased catches in fall 1984 and 1985 in the stocked bay were primarily in the 7.6-cm stretched mesh,



#### Figure 2

Mean catch rate (no./h) of red drum in gill nets in the Corpus Christi Bay (stocked) and upper Laguna Madre (unstocked) systems in fall (Sept-Nov) and spring (April-June) during 1983-86. The historic mean fall catch rate (for the period 1975-83) is also shown. Stocking occurred in fall 1983 (4.7 million fish) and January 1985 (250,000).

but this pattern did not occur in the unstocked bay (Fig. 3). This reflects recruitment of stocked fish to the 7.6-cm stretched mesh 1 year after each stocking. The stocked fish were evident in each subsequent season in the larger mesh portions of gill nets. In the San Antonio Bay system, the mean catch rate in gill nets decreased after the 1983 stocking, but increased after the 1984 stocking (Fig. 4). Apparently very few of the 2.3 million fish stocked in 1983 survived this freeze. The mean catch rate in gill nets in spring 1984 (0.2 fish/h) was among the lowest recorded since 1975 (Fig. 4). However, wild fish may have been more affected than stocked fish. The mean catch rate of age-I wild fish, those in the 7.6-cm stretched mesh part of the nets, declined to the lowest level ever (<1 fish/h), while catches in the larger meshes did not decline. Fish stocked in July 1984 were first apparent in gill nets in fall 1985, and the mean catch rate in gill nets was the third highest on record (Fig. 4). Most (83%) of this catch was in the 7.6-cm stretched mesh.

Stocking apparently increased the fishing success of sportboat anglers for red drum. The mean landing rate by these fishermen increased 150% over the mean historic (1979-84) rate in the stocked Corpus Christi Bay system in the highuse (15 May-15 November) season of 1985 (Fig. 5) when stocked fish reached the minimum legal size limit of 457 mm. The mean landing rate also increased in the unstocked upper Laguna Madre, but by only 50%. The mean landing rate in the following low-use season (16 November-14 May) in 1985-86 was similar to the historic catch rate in the stocked



#### **Figure 3**

Mean catch rate (no./h) of red drum in the 7.6, 10.2, and 12.7 cm stretched-mesh portions of gill nets in the Corpus Christi Bay (stocked) and upper Laguna Madre (unstocked) systems, fall (Sept-Nov) and spring (April-June), during 1983-86. Stocking occurred in fall 1983 and 1984.

bay, but it declined in the unstocked bay. The number of red drum landed from the stocked bay also increased 100% over the historic mean in 1985-86 (Fig. 6). Landings in the unstocked bay increased only 27% from the historic mean, although anglers fished 45% more man-hours in the unstocked bay than in the stocked bay in 1985-86 (882 vs. 609 man-hours). The increased landings over the historic mean in the upper Laguna Madre (18,500 vs. 14,600 fish, respectively) would have been much less than 27% had the unusually low 1984-85 landings (3,800 fish) not been included in calculating the historic mean. Annual landings in all other years in the upper Laguna Madre were 13,100 to 25,700 fish and averaged 16,800 fish.





### Figure 5

Mean landing (catch) rate (no./man-hour) of red drum landed by sportboat anglers in the Corpus Christi Bay (stocked) and upper Laguna Madre (unstocked) systems before and after fish reached minimum size limits of 457 mm TL in the high-use (15 May-20 Nov) and low-use (21 Nov-14 May) season during 1979-86.

Stocking also benefited anglers in the San Antonio Bay system. Although the 1983-84 freeze greatly reduced the number of available fish, the anglers' mean landing rate increased from the 1982-83 low-use season in the San Antonio Bay system in that low-use season when stocked fish became retainable by anglers (Table 1). The mean catch rate also increased in the adjacent Matagorda Bay system; some fingerlings may have moved to this system. Mean landing rates in other bays either remained the same, decreased, or remained less than 0.1 fish/man-hour. Landings during the low-use 1984-85 season (5,800 fish) were about the same



Mean catch rate (no./h) of red drum in gill nets in the San Antonio Bay system, fall (Sept-Nov) and spring (April-June), during 1975-86. Arrows indicate dates of major events affecting red drum. Fish were stocked in spring 1983 (2.3 million) and in summer 1984 (6.0 million).



#### Figure 6

Number of red drum landed annually (15 May-14 May) by sportboat anglers in the Corpus Christi Bay (stocked) and upper Laguna Madre (unstocked) systems before and after stocked fish reached minimum size limit of 457 mm TL.

as the historic mean (6,200 fish) although effort (36,200 manhours) was about 50% less. Fish stocked in 1984 are just now becoming retainable by fishermen.

Wild stocks of red drum can be enhanced through stocking to provide improved fishing success. However, the degree of improvement depends on such factors as the carrying capacity of each system, the number of wild fish present before stocking, fishing pressure, harvest restrictions, and climatic events. The sale of red drum caught in Texas was prohibited 2 years before stocking the Corpus Christi Bay system. About 3 months after the first stocking, the Texas

Table 1
Mean catch rate (no./man-hour) of red drum landed by private sport-boat anglers in Texas bay systems
during the low-use (21 Nov-14 May) seasons, 1982-83 and 1983-84.

Year	Galveston	Matagorda	San Antonio	Aransas	Corpus Christi	Upper Laguna Madre	Lower Laguna Madre
1982-83	0.01	0.03	0.04	0.07	<0.01	0.01	0.05
1983-84	0.01	0.07	0.13	0.06	0.05	0.01	0.04

coast experienced the worst freeze in history. The minimum size limit was increased from 406 to 457 mm, and the bag and possession limits were reduced by 50%. The existing and subsequently imposed restrictions reduced the impact of the freeze on red drum. Fishing improved in the upper Laguna Madre even without stocking; however, this improvement was even greater with stocking.

Additional research is needed to determine optimum stocking rates. An intensive stocking of fish marked with coded metal tags or genetically marked fish could be used to improve estimates of natural mortality rates and the contribution of stocked fish to the wild stocks and angler harvest.

These preliminary results are not based on a rigorous statistical analysis of the data collected. The landings data have not been adjusted for changes in minimum size limits or bag and possession limits. Therefore, the conclusions presented are conservative. A more rigorous analysis is planned after the 5-year project ends in 1988 and all stocked fish have left the estuarine fishery.

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# Genetic Marking and Ocean Farming<sup>1</sup>

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Department of Zoology Southern Illinois University Carbondale, Illinois 62901 Individuals from discrete subgroups within a species usually lack readily visible characters (or marks) that permit subgroup classification. More subtle distinguishing characteristics sometimes become apparent through specialized procedures, and various methods have been devised to impose identifiable attributes on individuals within a group (i.e., marking). Most commonly used marks are restricted to the immediate generation because they are largely or entirely not heritable.

Completely heritable characters, particularly allelic protein genes detected by electrophoresis, have proven to be valuable genetic marks. Previously unknown genetically isolated subgroups of many fishes have been identified (Allendorf et al. 1987). This information has been used to monitor migrations of distinct groups in stock mixtures (e.g., Milner et al. 1985). Natural genetic differences between populations have been used to estimate proportions of stocked and unstocked fish in specific fisheries (e.g., Murphy et al. 1983).

Intentional breeding using distinct genotypes (i.e., genetic marking) has been used to create identifiable groups. Experimental applications of genetically marked groups have included measurements of growth and survival of wild, hatchery, and hybrid steelhead in different environments (Reisenbichler and McIntyre 1977), quantifying contributions of chum salmon males of different behavioral states (Schroder 1982), and identifying differing fertilization rates of sperm from individual pink salmon males examined under varying conditions (Gharrett and Shirley 1985).

Genetically marked populations have also been created and monitored. Two concerns that must be met if the marked populations are to approximate the long-term performance potential of the parent stock are (1) a negligible effect on performance of individuals having different genotypes for the alleles involved in the marking process, and (2) an adequate sampling of genes over all loci from the parent stock in the marked population. Guidelines relating to the first concern are listed below:

1 Be particularly cautious of variants where good evidence for selection has been indicated in other organisms for particular protein classes.

2 Seek variants that occur widely and in diverse environments.

3 Be careful of rare alleles or those with substantial frequencies in restricted environments.

4 Monitor the performance of comparable marked and unmarked individuals and populations.

The second concern relates to the effective numbers of breeding individuals in the founding populations and in subsequent generations. Guidelines (suggested by Allendorf and Ryman 1987) include:

1 Use 25 individuals of each sex (with equal contributions from individual matings) as an absolute minimum for establishing a new population.

<sup>&</sup>lt;sup>1</sup>The information in this extended abstract is included in the following article: Utter, F.M., and J.E. Seeb. In press. Genetic marking in fishes: Overview focusing on protein variation. Trans. Am. Fish. Soc.

2 Use a considerably larger number of individuals for maintenance of established populations.

Two projects involving Pacific salmon species demonstrate somewhat different applications of genetically marking populations. A segment of a chum salmon run to a stream in Puget Sound, Washington, USA, was genetically marked for five consecutive years using males having selected genotypes for two enzyme systems mated with randomly chosen females (Seeb et al. 1986). Allele frequency differences between marked groups and the parent population (10% or greater for both enzyme systems) resulted in estimates of enhancement contributions to the total returning adult populations in the stream from 6% to 29%. Dilution of the mark in the adjacent inlet resulted in estimates between 2.2 and 4.3 million juvenile fish.

Late-returning segments of even- and odd-year runs of pink salmon were genetically marked for four consecutive years at a hatchery on a stream near Juneau, Alaska, USA (Lane 1984, Gharrett 1985); different single enzyme marks were used for even- and odd-year runs. Both males and females were selected for breeders, permitting a much larger change of allele frequency between the parent and marked populations. No straying to adjacent drainages was detected for either year-class. Evidence of straying within the drainage of the parent population was observed only for the late segment of the even-year run both upstream and downstream from the hatchery.

Genetic marking projects similar to those summarized above are feasible for any cultured population. Cultured marine species are particularly suitable for genetic marking; their generally reduced genetic divergence relative to freshwater and anadromous species (Gyllensten 1985) makes marked populations more readily apparent amidst a more uniform background. Genetic marking can also be extended to wild populations, providing potential breeders can be intercepted, genotyped, and only those of appropriate genotype permitted to spawn.

Genetic marking provides a heritable brand with diverse uses. Marked populations permit monitoring of intermingling and interbreeding with other populations. This capability relates to environmental and genetic concerns about intentional or accidental releases of cultured or transplanted populations. Conversely, genetically marked populations can be used by their owners or stewards for identification in mixed harvests.

Although protein coding genes are presently the most useful source of materials for genetic marking, they represent less than 1% of the total DNA of an organism. Much additional genetic variation exists that is potentially useful for genetic marking. The DNA of mitochondria is a source of variation that is finding increasing application as a population marker (e.g., Ferris and Berg 1987). Immunologically detected genetic markers are another souce of variation that may be useful. The same principles concerning the performance of genotypes and the adequacy of effective population size pertain to any genetic mark that is used.

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# Culture of North American Sturgeons for Fishery Enhancement<sup>1,2</sup>

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## ABSTRACT

North American sturgeons were important to early colonists, and about 1860 large-scale exploration was initiated. However, by the turn of the century, most sturgeon stocks were severely depleted and the major fisheries collapsed. Early fishery managers sought to rehabilitate the stocks through propagated fish, but suitable culture efforts could not be developed and efforts were abandoned by about 1910.

In recent years, protection of some sturgeon stocks has resulted from enactment of controlled fishing regulations and/or listing species as an endangered species. Renewal interest has focused on spawning and culture of most North American sturgeons, and today there are small-scale stocking efforts underway with several species. Sturgeons can be considered "living fossils," exhibiting little change from their sturgeon-like ancestors of the upper Cretaceous period, 100 million years ago. Worldwide, there are 25 species of sturgeons, 18 of the genus *Acipenser*, two of the genus *Huso* (which contains the largest sturgeon), two shovelnose sturgeons (*Scaphirhynchus*), and three of the genus *Pseudoscaphirhynchus*. In North America, there are eight species inhabiting various freshwater and/or coastal habitats (Table 1). Of these, Atlantic sturgeon *Acipenser* oxythynchus, lake sturgeon *A. fulvescens*, shortnose sturgeon *A. brevirostrum*, white sturgeon *A. transmontanus*, and paddlefish *Polyodon spathula*, have received substantial interest in recent years for purposes ranging from stock enhancement to commercial aquaculture.

Historically, sturgeons were important to early settlers and served as an item of commerce. Reports and books in colonial days often contained information on the abundance of these awesome creatures which the indians named "Mishe-Nahma" or "King of Fishes." Large-scale commercial exploitation of North American sturgeon stocks began during the last quarter of the 19th century. The rapidity with which the major stocks were depleted astonished fishermen and fishery managers. The statement by Tower (1909) typifies the thoughts and feelings of the time-"It seems scarcely comprehensible that a fish so widely distributed through the country, so abundant, and so little used less than three decades ago, has so rapidly disappeared that the end is already in sight." This overutilization of the sturgeons as well as other natural resources was responsible for the initiation of a conservation movement around 1907. Such conservation efforts, however, came too late to have any significant impact on the sturgeons.

Today, only remnant populations remain of most major North American stocks of sturgeons, and their geographic ranges have been sharply reduced from those of only 100 years ago. The purpose of this report is to briefly review the early and current sturgeon fisheries and to discuss the culture efforts for fishery enhancement of North American sturgeons, focusing on the more important Atlantic, lake, shortnose and white sturgeons, and the paddlefish.

## Exploitation of sturgeons \_

## **Early fisheries**

Utilization of North American sturgeons varied according to species, area, and time. During the early to mid-19th century, sturgeons were not highly regarded. At this time, they were intentionally killed to reduce damage to fishing nets, fed to livestock, used as fertilizer and to fuel boilers of steamboats (Harkness and Dymond 1961, Galbreath 1985). However, sturgeons eventually became highly prized and were valued as the most expensive freshwater fish. Large-scale exploitation began around 1860 when it was learned that smoked sturgeon could be substituted for smoked halibut and

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Table 1           Geographical distribution and general habitat of North American sturgeons.						
Species	Common name	Geographical distribution	Habitat			
Acipenser						
brevirostrum	Shortnose sturgeon	Atlantic coast from St. John River, New Brunswick, Canada, to St. Johns River, east coast of Florida	Anadromous; large coastal rivers			
fulvescens	Lake sturgeon	Mississippi River, the Great Lakes, and the Hudson Bay drainage basins	Freshwater; lakes and large rivers			
medirostris	Green sturgeon	Pacific coast from Gulf of Alaska south to North Baja, Cali- fornia, especially the Columbia River	Anadromous; primarily estuarine			
oxyrhynchus	Atlantic sturgeon	Atlantic coast from Labrador through Gulf of Mexico to northern coast of South America	Anadromous; primarily estuarine			
transmontanus	White sturgeon	Pacific coast fom Gulf of Alaska south to north Baja, Cali- fornia, especially Columbia River and Sacramento-San Joaquin system	Anadromous or semi-anadromous; large flowing rivers			
Scaphirhynchus						
albus	Pallid sturgeon	Mississippi River from Illinois south to Louisiana, Missouri River from Montana to Missouri	Freshwater; large turbid flowing rivers			
platorhynchus	Shovelnose sturgeon	Ohio, Mississippi, and Missouri Rivers; Mobile Bay drain- age, Alabama River, Rio Grande in Texas and New Mexico	Freshwater; large turbid flowing rivers			
Polydon spathula	Paddlefish	Mississippi River system, Mobile Bay drainage, Alabama River west to east Texas	Freshwater; backwaters, sluggish pools, bayous, oxbows of large rivers and lakes			

that the eggs could be made into high-quality caviar. Besides these products, isinglass was derived from the swim bladder and cartilagenous backbone of sturgeons and used to clarify liquids and stiffen jams and jellies; fish oil could be rendered from the flesh and used in paints; and leather was made from the skin. However, the main products were the flesh and the caviar, as is the case today.

Generally, sturgeon fishing occurred during the spring as adults migrated to freshwater spawning areas, although some species, such as the white sturgeon, were harvested on their feeding grounds as well. A variety of gear was employed, including harpoons, grapple hooks, baited and unbaited fish hooks, and pound nets, trammel nets, weirs, stake row nets, seines, and gill nets. Such gear was quite effective on the highly susceptible sturgeons, and in relatively short periods of time, usually 5-10 years, major stocks of sturgeons became substantially depleted.

Landings from the various sturgeon fisheries differed somewhat, but the pattern of exploitation was always similar (Fig. 1). Landings increased rapidly over a relatively short period during initial exploitation, then declined sharply and remained at low levels. Primary fishing emphasis was on the Atlantic sturgeon (including the much smaller shortnose sturgeon), the white sturgeon, and the lake sturgeon. Landings of Atlantic sturgeon peaked about 1890 with landings of 3.3 million kg, but by the turn of the century all major fisheries exhibited substantial declines or total collapse (Murawski and Pacheco 1977, Smith 1985). In 1892, a peak production of about 2.5 million kg of white sturgeon was recorded from the Columbia River. However, by 1899 the fishery had collapsed and landings were only about 50,000 kg (Galbreath 1985). Similarly, landing of lake sturgeon peaked around 1885 (2.3 million kg smoked flesh; 1,000 kegs caviar; 1,400 kg isinglass) and then suffered a similar decline (Harkness and Dymond 1961). Commercial harvesting of paddlefish became important after the lake sturgeon stocks were depleted. By 1899, landings of paddlefish had increased to 1.1 million kg from 0.47 million kg in 1894. Like the Acipenseridae, paddlefish landings decreased shortly after large-scale exploitation began, but the decline was not as precipitous nor as drastic as that of the other sturgeons (Gengerke 1986). In 1922, landings of paddlefish were still 0.63 million kg, and over the next 43 years landings ranged from 0.27 million kg (1960) to 0.43 million kg (1975).

# **Current fisheries**

Today, commercial harvesting of some North American sturgeons still occurs; however, landings in the recreational fishery often exceed commercial landings. The Atlantic sturgeon has a broad geographical range, yet commercial harvesting is currently restricted to Canada, New York, North Carolina, and Georgia, where only nominal landings are reported (Smith 1985). Formerly, landings in South Carolina were substantial relative to total U.S. landings, but in recent years the fishery suffered major declines (Smith et al. 1984) resulting in an indefinite closure of the fishery. All existing Atlantic sturgeon fisheries in the United States should be closed to protect the remaining stocks. Harvesting of the sympatric shortnose sturgeon in the United States has been banned since 1972 when it was listed as an endangered species by the U.S. Fish and Wildlife Service (Miller 1972).



Figure 1 Commercial exploitation of various stocks of sturgeons. Data from (A) Murawski and Pacheco 1977, (B) Harkness and Dymond 1961, and (C) Galbreath 1985.

Similarly, the lake sturgeon is classified as rare over much of its original range by the U.S. Fish and Wildlife Service. However, this species does support a number of sport fisheries, none of which exceeds that in Lake Winnebago, Wisconsin (Folz and Meyers 1985). Harvesting of lake sturgeon was prohibited from 1916 to 1931, but in 1932 a spear fishing season was established on Lake Winnebago. Initially, spear fishermen were allowed to harvest 5 sturgeon per season, with a minimum length of 76 cm TL, but current regulations are more restrictive with only one fish per season of a 114-cm minimum length allowed. Fishing success rate varies from 0.4 to 32.8% and averages 13.2%. During 1955-83, harvests ranged from 8 to 2235 fish (1982) (Folz and Meyers 1985). Based on harvest data and sampling of spawning fish, it appears that the lake sturgeon stock in Lake Winnebago has not declined since 1955 and that the population is stable or increasing.

Landings of white sturgeon from the lower Columbia River (below the Bonneville Dam) in Washington and Oregon are now at their highest level since the turn of the century. Most commercial landings result from incidental capture in salmon gillnets, although there is some focused fishing for sturgeon in certain areas. Recreational hook-and-line fishing for white sturgeon in the lower Columbia River has been increasing steadily, and since 1977 recreational landings have exceeded commercial landings. From 1977 to 1983, average commercial landings have been 12,600 fish as compared with 27,300 fish for the recreational fisherman (Galbreath 1985). In terms of total number of fish caught, the landings in 1983 exceed the recorded peak landings in 1892. However, individual fish weight has declined from a former average size of 68 kg to a present weight of 14-16 kg in the commercial fishery and 8 kg in the sport catch (Galbreath 1985). Continuing research has established that successful spawning is occurring below Bonneville Dam, and the consensus is that the white sturgeon stocks in the lower Columbia River below the Dam are healthy. A combination of harvest regulations (minimum and maximum size limits), increased food supplies, and a shorter salmon gillnet fishing season are primarily responsible for the good condition of the sturgeon stocks. Some harvesting of green sturgeon does occur in conjunction with the white sturgeon, but their numbers are low and these fish are not highly regarded as a food fish. In the upper Columbia River (above the Bonneville Dam), white sturgeon are essentially landlocked within each dammed river segment or pool. Recruitment and stock size appears healthy in some areas, but declines are occurring in others. Additional research is needed to assess these various landlocked populations of white sturgeon.

In California, commercial harvesting of white sturgeon has been prohibited since 1917 but the sport fishery was reopened in 1954. Up to 1963, sturgeon were taken incidentally to fishing for striped bass, Morone saxatilis. However, in 1964 angler success improved dramatically with the use of shrimp (Crangon spp., Palaemon macrodactylus) as bait (Kohlhorst 1980). Since then, sturgeon have become the focus of an important sport fishery in the Sacramento-San Joaquin river system. Population estimates suggest that abundance in this system decreased between 1967 and 1974, but abundance has continually increased since then. These changes in population size are believed to be due to variable recruitment rather than to fishing pressure. Currently, it is estimated that there are about 130,000 legal-sized adult white sturgeon inhabiting this system, of which about 8% are harvested annually (David Kohlhorst, Calif. Dep. Fish Game, Stockton, CA, pers. commun., 29 Sept. 1986). No snag fishing is allowed in California and there is a minimum fish size of 102 cm (weight  $\sim$ 5.4-6.8 kg). Average size of the sport fish landed is 13-18 kg. Catch records from sturgeon charter boats indicate that between 1964 and 1983 the number of anglers/ year ranged from 1235 to 8284, and the number of fish caught per year ranged from 320 to 2272 (David Kohlhorst, Calif. Dep. Fish Game, Stockton, CA, pers. commun., 29 Sept. 1986). Landings by charter-boat anglers represent

	N	Recorded ma	Recorded maximum			
	Matu				Size	
Species	Age and sex (yr)	Size (cm TL)	Age (yr)	(cm TL)	Wt. (kg)	
Acipenser						
brevirostrum	9-14 (F) <sup>a</sup> 8-12 (M) <sup>a</sup>	57.2-73.3ª 64.2ª	57 <sup>b</sup>	143.0 <sup>b</sup>	23.6 <sup>b</sup>	
fulvescens <sup>c</sup>	24-26 (F) 14-16 (M)	139.7 114.3	152	240.0	140.9	
oxyrhynchus	7-19 (F) <sup>d</sup> 5-13 (M) <sup>d</sup>	173.0-234.1 <sup>d</sup> 124.6-185.7 <sup>d</sup>	60°	426.7 <sup>f</sup>	368.6 <sup>f</sup>	
transmontanus	15-20 (F) <sup>g</sup> 12 (M) <sup>g</sup>	168.0-183.0 <sup>g</sup> 122.0 <sup>g</sup>	>100 <sup>f</sup>	>610.0 <sup>f</sup>	675.0 <sup>h</sup>	
Polyodon spathula	8-14 (F) <sup>i</sup> 2-9	148.6-162.8 <sup>i</sup> 66.8-117.3	30 <sup>j</sup>	220.0 <sup>j</sup>	90.7 <sup>j</sup>	
<sup>a</sup> Taubert 1980 <sup>b</sup> Dadswell 1979	<sup>c</sup> Prelegel and Wirth 1977 <sup>d</sup> Smith et al. 1982	<sup>e</sup> Magnin 1964 <sup>f</sup> Scott and Crossman 1973		eath 1985 eath 1979	<sup>i</sup> Russell 1986 <sup>j</sup> Boschung et al. 1983	

only a fraction of the sturgeon caught and recent total annual catch is estimated to be about 10,000 fish.

In Idaho, there are catch-and-release sport fisheries for white sturgeon in the Snake and Kootenai Rivers. However, recent findings suggest that possible closure of several sections of the Snake River may be needed because of estimated low population size (Cochnauer et al. 1985).

In contrast to most Acipenseridae, some populations of paddlefish have actually increased substantially since the turn of the century, although other stocks no longer inhabit former ranges (e.g., Canadian stocks). In the Mississippi, Missouri, Ohio, and Red Rivers, paddlefish populations have significantly decreased while increased abundance has been reported from the Tennessee, Cumberland, and Arkansas Rivers (Gengerke 1986). Sport fisheries, based almost exclusively on snag fishing, are permitted in 17 states and provide landings equal to about 70% of the commercial landings. Annual harvest rates from sport and commercial fishing on the order of 15-20% do not appear to damage most populations (Pasch and Alexander 1986).

## **Reasons for decline**

As is evident from the landings data, sturgeons are highly susceptible to man's activities, despite their large size and extended life span (Table 2). Sturgeons mature at an advanced age (8-25 years), demonstrate protracted spawning periodicities (2-8 years), and inhabit areas of concommitant use by man (rivers, lakes, estuaries, coastal environments). Consequently, major population perturbations can be easily effected by man. In all cases, major stocks of sturgeons were overexploited by fishing (Harkness and Dymond 1961, Priegel and Wirth 1977, Galbreath 1985, Pasch and Alexander 1986, Smith 1985). Additionally, installation of dams on historic spawning rivers and widespread industrial pollution caused elimination or reduction in suitable sturgeon habitat (Harkness and Dymond 1961, Leland 1968).

# Fishery enhancement \_

# Early stock replenishment efforts

Near the end of the 19th century, fishery managers realized that the sturgeon fisheries had experienced substantial declines and that something would have to be done to restore the stocks if the fisheries were to be maintained. Unanimous agreement was reached to rehabilitate the various stocks through artificial propagation programs (Ryder 1890, Cobb 1900, Stone 1900). The first successful spawning of a North America sturgeon was accomplished on the Hudson River in 1875 by Seth Green and Aaron Marks with the New York State Fish Commission. Working with Atlantic sturgeon fishermen, eggs and milt were removed from ripe fish and artificially mixed. Using this approach, about 100,000 young were hatched over a two-week period. This early success led to the mistaken belief that sturgeon propagation would be an easy task. In 1888, the U.S. Fish Commission began spawning activities with the Atlantic sturgeon on the Delaware River under the direction of J.A. Ryder (Ryder 1890). Some limited successes occurred, but obtaining adequate numbers of ripe females was a problem. Further, fungal infestation by Achlya and Saprolegnia often caused loss of the incubating eggs. Subsequent workers attempting to spawn Atlantic sturgeon encountered similar problems of limited availability of ripe broodstock and fungal infections of eggs (Dean 1894, Meehan 1909, Leach 1920). Early spawning efforts with lake sturgeon also had limited success, although 5 million fry were produced in 1891 and released in the Detroit River by the Ohio Game and Fish Commission. Efforts continued, but successes were limited to instances in which running ripe females containing ovulated eggs were captured at the same time as ripe males (Harkness and Dymond 1961). Unfortunately, such instances were uncommon. During 1906-09, efforts were initiated to spawn the much smaller shortnose sturgeon. This work was conducted at the Torresdale Hatchery in Philadelphia where ponds were used in an attempt to naturally ripen captive adult shortnose sturgeon (Meehan 1909). In several instances, females expelling eggs were removed from the ponds and small numbers of fry were hatched. As before, acquisition of simultaneously ripe males and females was a problem.

In spite of the high level of interest, efforts to propagate sturgeons in the United States were abandoned by 1912. A short time later, Canadian workers initiated culture efforts but they also experienced the same problems as previously noted. About 1920, they discontinued their propagation efforts.

## Soviet propagation efforts

The demonstration that secretions of the pituitary gland could be used to induce final ripening of fish gonads (Atz and Pickford 1959) led to renewed interest in sturgeon propagation, especially in the Soviet Union where overfishing and installation of dams had caused declines in sturgeon populations similar to those in North America. Beginning in the early 1960s, a major propagation effort was initiated in the Soviet Union based on the use of extracted sturgeon pituitary glands to induce spawning of ripe sturgeons (Manea 1969). Annual production of stockable-size fingerling (1-3 g) is now approximately 60-100 million fish. Sturgeon fingerlings are stocked in river deltas during the summer and recaptured as sexually mature adults after 10-20 years of grow-out in the sea (Doroshov and Binkowski 1985). No sea fishing for sturgeon is allowed, and caviar is the main product of this sea ranching approach. Based on a survival rate of only 1-3%, the annual sturgeon landings in the late 1970s from the Caspian Sea basin was 26,000 mt (metric tons), which resulted in the production of 1750 mt of caviar (Doroshov and Binkowski 1985).

## **Current North American culture efforts**

During the past 10 years, there has been renewed interest in the culture of North American sturgeons. Information on the life history and ecology of the various species, coupled with the use of hormones, has resulted in the spawning of Atlantic sturgeon (Smith et al. 1980), shortnose sturgeon (Buckley and Kynard 1981, Smith et al. 1985), lake sturgeon (Avelallemant et al. 1983, Folz et al. 1983, Czeskleba et al. 1985), and white sturgeon (Doroshov et al. 1983). Techniques for spawning and rearing paddlefish were developed in the mid-1960s and early 1970s, and recently there have been efforts to rear this species for stocking purposes and for caviar production (Graham et al. 1986). Spawning tech-

niques for all species are still in the "art" stage rather than being a science, and success is highly dependent upon the condition and stage of ripeness of wild-caught broodstock. Of the above species, collection of Atlantic sturgeon broodstock is the most difficult as population numbers are low and spawning areas are poorly known and occur in deep areas of fast moving waters. Further, Atlantic sturgeon do not feed during their spawning migration and thus are not susceptible to hook-and-line capture. Consequently, only limited spawning and culture success has been obtained with Atlantic sturgeon despite substantial efforts undertaken in South Carolina (Smith et al. 1981, Smith and Dingley 1984). In contrast, lake sturgeon can be routinely observed in the act of spawning in areas where the current is upwelling and where large rocks, boulders, and broken slabs of concrete have been riprapped at a steep angle into the water (Priegel and Wirth 1977). Spawning females are dip-netted, and the free-flowing eggs are removed through a small incision. The female is sutured and returned to the water. Similarly, running ripe males can be dip-netted and their milt stripped by abdominal compression and used to fertilize the eggs (Czeskleba et al. 1985). Ripe white sturgeon are captured primarily by baited hook-and-line as they move into spawning areas in the Sacramento River. These fish are induced to spawn using hormonal injection (usually commercially available carp pituitaries). Additionally, success has been obtained in using selected prespawning white sturgeon collected in San Francisco Bay in the fall. Final maturation was induced in the spring and the fish were successfully spawned (Doroshov et al. 1983). Shortnose sturgeon are listed as an endangered species in the United States; therefore, a federal permit is required to collect them. In South Carolina, mature migrating broodstock are obtained from commercial shad fishermen as incidental catch in their gill nets. Like the white sturgeon, these fish can be induced to spawn by injection of fish pituitaries (Smith et al. 1985). Paddlefish are captured with gill nets within 1-11/2 months of their normal spawning time and held in hatchery tanks. They are induced to ovulate using paddlefish pituitary glands, although recent work with LH-RHa suggests that this hormone may be an excellent substitute (Graham et al. 1986).

Techniques for fertilization and incubation of eggs are generally similar among the sturgeons. Eggs are removed from white sturgeon and lake sturgeon through an abdominal incision over a short period of time. In contrast, eggs are stripped from shortnose sturgeon and paddlefish at 20-60 minute intervals over a long period of time. Sperm is collected from the males with a syringe and usually diluted with water (1:200) just prior to fertilization to prevent polyspermy. Eggs and sperm are mixed for about 5 minutes and then a silt, mud, or clay suspension is added to inhibit adhesion of the eggs. Also, chemical treatments have recently been developed to eliminate the adhesiveness (Kowtal et al. 1986). The non-adhesive eggs are incubated in McDonald incubators (jars) for about 5-7 days at a temperature of 14-16°C. During incubation, eggs are gently rolled with upflow water. Upon hatching, the sac-fry swim up and out of the incubators and are collected in adjacent tanks. After about 10 days, the fry begin feeding.

Larval and juvenile rearing differs among the various sturgeons. White and shortnose sturgeon can be trained to accept soft-moist and dry diets and are typically reared in tanks in intensive systems. Survival rate to a small juvenile size ( $\sim$ 30 g, 3-4 months old) is about 15%. After this size is attained, mortality rarely occurs. Rearing of larval and juvenile lake sturgeon has been difficult because they appear to require live foods (Anderson 1984, Czeskleba et al. 1985, Graham 1986a) and attempts to rear this species in fertilized ponds has resulted in poor survival. Thus, it is costly to produce large numbers of juvenile lake sturgeon. Paddlefish juveniles have been reared both extensively in ponds and intensively in tanks (Graham et al. 1986). In the extensive approach, ponds are fertilized to induce a dense zooplankton population which serves as food for paddlefish. Ponds are usually stocked when fry are 5 days old and at a density of 49,400 fish/ha. Average survival is 35% and growth is rapid. At the end of a 140-day growing season, most paddlefish are about 250-300 mm in length. In the intensive systems, larvae are initially reared on zooplankton (primarily Daphnia) and then trained to accept soft-moist and dry formulated feeds. Unfortunately, feeding is not efficient because paddlefish do not actively seek feed and they cannot be reared under crowded conditions. Although the intensive approach is successful, it requires a large amount of hatchery space.

## Stock enhancement programs \_\_\_\_

Although substantial progress has been achieved in rearing some of the more important North American sturgeons, efforts to enhance and/or reestablish fisheries are relatively small scale. The culture technology for white sturgeon is by far the most developed. There are a number of aquaculture operations in California growing this species as a food fish (Ken Beer, The Fishery, Galt, CA, pers. commun., 2 Oct. 1986). Further, development of cultured broodstock has progressed well. At the University of California, Davis, 21/2-3 year-old cultured males have been successfully used to fertilize eggs from wild-caught females (Serge Doroshov, Univ. Calif., Davis, CA, pers. commun., 17 June 1986). Further, 5-year-old females are beginning to show signs of maturation. Commercial sturgeon farmers in California now routinely use cultured males and are rearing females in hopes of eliminating their dependency on wild fish. In spite of this well-developed hatchery technology, there currently are no plans for stocks enhancement programs for white sturgeon, because fishery managers feel that most stocks are stable or increasing. However, the sturgeon farmers are required by their collecting permits to restock sevral thousand sac-fry per wild sturgeon used for spawning purposes.

On the Atlantic coast, there is substantial interest in establishing stocking programs for the Atlantic and shortnose sturgeons. However, difficulties in capture of Atlantic sturgeon broodstock has thus far prevented initiaion of any stocking programs with this species. During 1986, only 26 adult Atlantic sturgeon were captured during an 8-week intensive fishing effort by ex-commercial sturgeon fishermen. Of these, no males were running ripe and no females could be induced to ovulate. In contrast to Atlantic sturgeon, significant progress has been attained in spawning and culture of shortnose sturgeon. Basic spawning techniques have been developed and fry have been produced during 1984-86. Grow-out of juveniles in intensive systems has been successful (Smith et al. 1986), and some fish have attained a mature size (1.8-2.1 kg) after only 18 months of culture. Thus, development of domesticated broodstock for the species appears promising. In 1985-86, a total of 6600 juveniles were released in South Carolina waters, of which 541 were 35 cm in size and were tagged. Preliminary capture data suggest these fish are surviving and growing in the wild, but a number of basic questions need to be addressed. Among these are questions concerning homing behavior, optimum size of juveniles for release, and size of natural populations. Studies are underway in South Carolina to examine these and other questions which will help determine the feasibility of stock replenishment programs with shortnose sturgeon.

There has been interest in stocking programs with the lake sturgeon in several areas of former abundance. The Menominee River, which forms a boundary between the upper peninsula of Michigan and northeastern Wisconsin, has several dammed sections that support fishable populations of lake sturgeon. In 1982, 290 large juveniles (18 cm) and in 1983, 11,000 small juveniles (30 mm) were stocked in the Sturgeon Falls section of the Menominee River, a site uninhabited by sturgeons in recent years (Thuemler 1985). The area appeared suitable as sturgeon habitat, but subsequent sampling efforts and radio telemetry studies indicated that the stocked fish moved out of that section of the river (Dan Folz, Wisc. Dep. Nat. Resourc., Oshkosh, WI, pers. commun., 18 June 1986). There is speculation that the Lake Winnebago strain of fish used to stock this area did not possess the needed behavioral characteristics of the "river race" of lake sturgeon that inhabit the Menominee River. At present, the Lake Winnebago population of lake sturgeon is stable or increasing, and additional spawning sites are being documented in the Wolf River resulting from installation of additional riprapping of the shoreline accompanying increased development. Thus, Wisconsin's main focus is to refine culture techniques in anticipation of future need (AveLallemant et al. 1983) and to continue to intensively manage the existing population in Lake Winnebago to maintain a sustained yield (Folz and Meyers 1985). However, there are 4-6 locations in Wisconsin formerly containing sturgeon populations that appear to be environmentally suited for restocking. Proposed restocking protocol suggest that 3 years of consecutive stocking should be undertaken using fry, fingerlings, and adults from the same river systems, if possible, to preserve the genetic integrity of the stocks.

In Missouri, initial efforts are underway to implement a lake sturgeon reintroduction plan (Graham 1984). Lake sturgeon were once abundant in Missouri, but now there are only isolated reports of occasional individuals being caught in the Missouri and Mississippi rivers. Graham (1984) evaluated the characteristics of the various state waters and has identified numerous rivers, lakes, and reservoirs that appear suitable for restocking of lake sturgeon. To test the feasibility of the restocking plan, Mark Twain Lake in northeastern Missouri was stocked in 1984 with 11,800 culture fingerlings originating from Lake Winnebago sturgeon (Graham 1986a). This new reservoir was selected because it contained abundant natural food, few predators, and turbid water. Additionally, a main tributary appeared to offer suitable spawning conditions. No sampling of the 7900-ha reservoir has been attempted, but one fish was reported captured in early summer 1986. During the fall 1986, an additional 10,750 fingerlings (~200 mm in size) were stocked into this reservoir to complete the stocking program. Reintroduction success will be evaluated over time, and, depending on the results of this stocking program, other waters in Missouri may be similarly stocked. The feasibility of restocking lake sturgeon in selected waters in Minnesota is also under examination.

Several midwestern states have considered stocking paddlefish, but lack of a dependable supply of fingerlings has delayed stocking efforts. However, from 1970 to 1977, paddlefish were stocked in Table Rock Lake, a 17,500-ha lake in southwestern Missouri previously uninhabited by this species (Graham 1986b). Paddlefish fry (6-8 mm) were stocked in 1970, but population analyses suggest that these fish did not survive. Between 1972 and 1977, 82,600 (250-300 mm) fingerlings were stocked and these fish appeared to have an excellent survival rate. Growth of the introduced fish has been rapid and there was evidence of spawning in 1983. As a result of this stocking program, an expanding sport fishery (by snagging) has developed, with 2970 fish caught during 1983-84. In 1984, the estimated fishery landings were 18,000 kg, the most successful paddlefish stocking program to date. Missouri is stocking fingerlings in Lake of the Ozarks to maintain a fishery and also into Harry S. Truman Reservoir in an attempt to establish a population. In 1983, Alabama stocked a 104-ha lake with 440 fish (0.5 kg in size) in the hopes of eventually harvesting these fish for food. Additionally, the Kansas Game and Fish Commission stocked about 5000 paddlefish juveniles (10-50 cm) into the 3600-ha John Redmon Reservoir in an attempt to establish a population (Graham 1986b). Besides the interest to establish fisheries through stocking programs, there is also interest in culturing paddlefish as an aquaculture species (Semmens and Shelton 1986). However, development of aquaculture technology for this species is still in the preliminary stage.

## **Conclusions**.

Significant progress has been achieved in recent years in propagating various North American sturgeons. In some cases, culture technology is sufficiently advanced to provide the basis for development of an aquaculture industry (e.g., white sturgeon; Marx 1986). However, for the most part, stock enhancement or reintroduction programs are still in the conceptual or preliminary stocking-assessment phase for most species. In many instances, availability of suitable quantities of stockable juveniles is the problem, while in other cases the sturgeon resources have been so depleted that state management agencies have prior commitments to maintain existing fisheries and, therefore, are unwilling to commit the resources needed to develop stock enhancement programs. Sturgeon populations cross many state boundaries and historically they covered broad expanses of North America. Thus, it is proposed that cooperative state-federal programs be jointly sponsored, perhaps through regional agreements. To a certain degree, the present progress achieved in culture of North American sturgeons (e.g., white, shortnose, lake) is in part attributable to support from various federal agencies (e.g., U.S. Fish and Wildlife Service, Sea Grant Office of the Department of Commerce). Since sturgeon mature and spawn at an advanced age, a long-term commitment by the various states and federal agencies will be required to properly evaluate the potential for stugeon restoration efforts. Unfortunately, such commitment does not appear likely from any governmental entity at present. For the foreseeable future, stock enhancement programs will continue on a modest scale. Planning and recommendations for stocking programs and their evaluation as proposed by the states of Missouri and Wisconsin are commendable and should serve as an example to other states and agencies. With proper foresight, the programs underway today could well result in a higher level of interest and activity by fishery managers in the future.

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# Application of Yield-per-Recruit and Surplus Production Models to Fishery Enhancement Through Juvenile Releases

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## ABSTRACT

Yield-per-recruit and surplus production models are modified for use when hatchery-reared juveniles are released into a fishery. The yield-per-recruit model indicates that species with low ratios of natural mortality to growth and high asymptotic weight offer the greatest potential weight yield per stocked juvenile. The Ricker surplus production model is easily modified to express catches as functions of fishing effort and numbers of juveniles released. Thus the model can be used to estimate the effectiveness of stocking a fishery with hatchery releases based on a time series of catch, stocking, and effort data. The model can also be used as a simulation tool. The release of hatchery-reared juveniles to enhance fisheries for a number of species is practiced in Japan and to a lesser degree in other countries, including the United States and Norway (Yatsuyanagi 1982, Botsford and Hobbs 1984, Isibasi 1984, Ulltang 1984). When juveniles are released to augment a natural stock that is the basis of an existing fishery, stocking combines extensive mariculture with traditional fishery science. New quantitative tools are needed to evaluate and manage this system. The simple stocking vs. harvesting ratios that are used to evaluate and manage aquaculture no longer apply in the presence of varying fishing pressure and a natural stock. For example, fishing mortality often increases as stocking levels increase, making it difficult to attribute any increase in yield solely to the increase in stocking. However, traditional fishery production models are also inadequate since they do not incorporate stocking as a variable. Although some models have been developed to examine the effects of stocking relative to hatchery cost and the return to the fishery, these analyses have not taken into account other management variables including size limits and fishing effort (e.g., Oshima 1984). More sophisticated models have been developed which can be used to simulate the effects of stocking or develop optimal fishery policy, and can be applied in situations where the biology of the resource is well known and estimates of age-specific population parameters are available (Watanabe et al. 1982, Botsford and Hobbs 1984, Ulltang 1984, Watanabe 1985).

In this paper the traditional Beverton and Holt (1957) yieldper-recruit model and the nonequilibrium Ricker surplus production model (Ludwig and Walters 1985), both standard tools for fishery management, will be modified so that they can be applied to evaluate and manage fisheries in which juveniles are released. The yield-per-recruit model can be applied with very little modification to juvenile releases to evaluate the yield-per-released-juvenile as a function of the biological parameters (growth and mortality) and management parameters (release size, size at entry, and fishing mortality). The contribution of the released juveniles to the spawning stock can be evaluated in a similar fashion by computing the spawning-stock biomass per released juvenile. The Ricker surplus production model can be modified to express the catch as a function of effort and stocking so that a timeseries of stocking, catch, and effort data can be analyzed to evaluate the effectiveness of stocking and to estimate maximum sustainable yield in the presence of juvenile releases.

# Yield-per-recruit models \_\_\_\_\_

The Beverton and Holt (1957) yield equation can be formulated as a function of the ratio of instantaneous mortality to von Bertalanffy growth (M/K), the ratio of length at recruitment to the fishery to asymptotic length (c), the ratio of fishing mortality to natural mortality (F/M), and the ratio of length of the stocked juvenile to the asymptotic length (a).



Figure 1 Yield per stocked juvenile for *Pristipomoides filamentosus* as function of relative length of entry and relative fishing mortality. Estimates of M/K = 1.7,  $W_{\infty} = 8.5$  kg taken from Ralston (1981); size of release taken as 0.1  $L_{\infty}$ .

Under this formulation the yield (Y) per stocked juveniles (S) is:

Y/S =

$$\begin{split} &(M/K) (F/M) ((1-c)/(1-a))^{(M/K)} (1/(M/K + (F/M)(M/K))) \\ &- 3(1-c)/(1+(M/K) + (F/M)(M/K)) \\ &+ 3(1-c)^2/(2+(M/K) + (M/K)(F/M))) \\ &- (1-c)^3/(3+(M/K) + (F/M)(M/K)). \end{split}$$

In a similar fashion, the spawning-stock biomass can be expressed as a function of the same variables plus the ratio of the length at onset of sexual maturity to the asymptotic length (Beddington and Cooke 1983).

Based on these formulations, just as in the traditional yieldper-recruit analysis, the yield-per-stocked juvenile (Y/S) and the contribution of the stocked juvenile to the spawning stock biomass (SSB/S) can be calculated as functions of F/M and c (Figs. 1,2). The value of the yield per stocked juvenile varies considerably with c and F/M, so the proper choice of F/M and c is necessary to maximize the benefit from stocking. For example, in Figure 1, when the length of entry to the fishery is 50% of the asymptotic length, a hatcheryreleased juvenile opakapaka, Pristipomoides filamentosus, contributes 0.3 kg to the fishery when fishing mortality equals natural mortality; whereas when fishing mortality increases to 1.5 natural mortality, at the same size of entry, the contribution to the fishery of a hatchery-released juvenile opakapaka will increase 33% to 0.4 kg. The SSB/S isopleths indicate the contribution of a stocked juvenile to the population spawning-stock biomass. For example, for the snapper (opakapaka) when F/M = 1.5 and c = 0.5, a stocked juvenile will contribute 0.15 kg to the population spawning-stock biomass (Fig. 2). If the spawning-stock biomass of the population is known, the SSB/S equation can estimate the number of juveniles needed to be released to increase the



## Figure 2

Spawning-stock biomass (kg) per stocked juvenile for *Pristipomoides filamentosus* as function of relative length of entry and relative fishing mortality. Estimates of M/K = 1.7,  $W_{\infty} = 8.5$  kg taken from Ralston (1981); size of onset of sexual maturity taken as  $0.5 L_{\infty}$ ; size of release set at  $0.1 L_{\infty}$ .

population spawning-stock biomass to a given level. The SSB/S and Y/S equations, together with the hatchery costs and the value of the harvested fish, can serve to evaluate the economic benefits of the release programs as functions of variables c, a, M/K, and F/M.

Hatchery technology and knowledge of the early-life history of marine organisms have made it possible to rear numerous marine organisms. The Y/S and SSB/S equations permit comparisons of the benefits from stocking among species with different population parameters. For example, there are three commercially important species in Hawaii that might be candidates for hatchery release programs: Mahimahi, Coryphaena hippurus; a snapper, P. filamentosus; and a spiny lobster, Panulirus marginatus. The Y/S isopleths were computed for each of these species, and the maximum values of Y/S, for all c, as a function of F/M, were determined. These maximum values of Y/S are plotted for the three species (Fig. 3). The differences between the three species in their contribution to the fishery are striking. For example, when fishing mortality is equal to natural mortality and the size at entry to the fishery is optimal, a released spiny lobster will contribute 0.02 kg to the fishery, a released snapper will contribute 0.3 kg, and a released mahimahi will contribute an amazing 2.5 kg. Even when price per kilogram is considered and the possibility that only 25-50% of adult mahimahi remain around the islands, the mahimahi releases appear to offer high economic return. The contribution of a released mahimahi to the fishery is so much greater than that of the snapper, and in turn the contribution of a snapper is greater than that of the spiny lobster, largely because of differences in the M/K ratio (1.0 for mahimahi, 1.7 for snapper, and 3.0 for lobster) and the asymptotic weight (30 kg for mahimahi, 8.5 kg for snapper, and 1.7 kg for spiny lobster). The lower the ratio of natural mortality to growth, the greater the survival of the released individual; and the


### Figure 3

Maximum yield per stocked juvenile as a function of relative fishing mortality for mahimahi, opakapaka, and spiny lobster. Parameter estimates for mahimahi M/K = 1.0, W = 30 kg (Uchiyama et al. 1986); for opakapaka M/K = 1.7, W = 8.5 kg (Ralston 1981); for spiny lobster M/K = 3.0, W = 1.7 kg (Polovina unpubl. data).

greater the asymptotic weight, the greater the weight gained by the released individual. The *SSB/S* follows the same order for the three species as Y/S. Thus among the candidates for juvenile release, those with low M/K ratios and high asymptotic weights will offer the greatest contribution in biomass to the fishery.

# Fishery production models with stocking \_\_\_\_\_

The most frequently used production models, Schaefer and Gulland-Fox, do not explicitly specify a recruitment relationship, and hence do not easily lend themselves to modification to include hatchery releases. However, the Ricker model for surplus production (Ludwig and Hilborn 1983) is a simple production model which can easily handle stocking. The Ricker model for surplus production is expressed by the following three equations (Ludwig and Walters 1985):

$$B_{t+1} = S_t \exp(A - BS_t + U_t) \tag{1}$$

$$S_t = B_t - C_t \tag{2}$$

$$C_t = B_t (1 - \exp(-qE_t)) \tag{3}$$

where  $B_t$  is the population biomass in year t,  $S_t$  is the biomass remaining after harvest in year t,  $C_t$  represents the catch in year t,  $E_t$  denotes the effort in year t,  $U_t$  represents independent normally distributed random variables with mean 0 and variance v, and A, B, and q are parameters estimated from catch and effort data.

To modify these equations to include  $H_t$  hatchery-released juveniles in year t before harvesting, it is necessary to express the biomass in year t + 1 resulting from  $H_t$ . A power function relationship

$$B_{t+1} = a(H_t)^b$$

with parameters a and b appears appropriate for hatchery releases of Oregon coho salmon (Peterman and Routledge 1983). For a fast-growing species the major contribution from stocking to the fishable biomass will occur in the same year as the stocking, and thus  $H_{t+1}$  rather than  $H_t$  would be used in the power function equation.

If the biomass from the hatchery-released stock is simply added to that of the natural stock in the first equation of the Ricker model for surplus production, then we obtain:

$$B_{t+1} = S_t \exp(A - BS_t + U_t) + a(H_t)^b.$$
 (4)

This modified equation, together with the two other equations of the Ricker model, produces a production model which incorporates hatchery releases. The contribution of the hatchery releases will increase the catch directly through Equation (3) and those that are not caught will increase  $S_t$ through Equation (2).

The Ricker surplus model without stocking shows the usual dome shape in which production first increases then decreases ultimately to zero with increasing fishing mortality (Fig. 4). When a fixed number of hatchery releases are added to the system, the yield curve has the usual dome shape as a function of fishing mortality; but rather than declining to zero, as is the case of an unstocked population, the yield approaches an asymptotic yield of  $a(H_t)^b$  with increasing fishing mortality (Fig. 4). The relative contribution of the releases to the fishery will be greatest for relatively high levels of fishing mortality. Hatchery releases can increase the maximum yield and the corresponding level of optimum fishing effort.

If hatchery releases occur in the absence of a natural population the Ricker model with stocking just reduces one equation:

$$C_t = a(H_t)^b (1 - \exp(-qE_t)).$$

Unfortunately, due to the nonlinear nature of the Ricker surplus production model, it is not as easy to estimate the parameters as, for example, for the Schaefer model. A complete approach to parameter estimation for the Ricker model is presented in Ludwig and Hilborn (1983). Here a simplified approach will be presented for the Ricker model with stocking when it is assumed that the fishing effort is measured without error. First, assume a value for q and compute  $B_t$ and  $C_t$  from Equations (2) and (3). Then estimate A, B, a, and b from Equation (4) with the nonlinear regression, using the B's and S's obtained from the previous step. Finally, vary q and repeat the previous steps until the sums of squares of the nonlinear regression can typically be used as a basis for this parameter estimation approach.



Equilibrium Ricker surplus production model with and without stocking. Curve without stocking based on biomass model  $B = S \exp(0.7 - 0.007S)$ ; curve with stocking based on  $B = S \exp(0.7 - 0.007S) + 25$ .

An experimental approach to stocking can be an efficient means of evaluating the effectiveness of stocking and identifying optimal stocking levels, but simulation of any design is a necessary first step before implementation. For example, releasing juveniles into a fishery on alternating years and then comparing catches in years with stocking to catches in years without stocking may be considered a way to estimate the effectiveness of stocking. This experimental design can be simulated with the stocking surplus production model (Fig. 5). Suppose a population has a carrying-capacity biomass of 100 t and is fished with a fishing mortality of F = 1.0. Suppose juveniles are released in a quantity which contributes 20 t to the fishable biomass over a 10-year period on years 2, 4, 6, 8, and 10, and no releases occur in years 1, 3, 5, 7, and 9. The stocking surplus production model estimates that equilibrium fishing with F = 1.0 results in a catch of about 49 t annually. The first stocking (year 2) increases the catch to 62 t, and then the catch follows an oscillating sequence of lower catches during years without stocking and higher catches during years with stocking. The oscillating sequence has an increasing trend over time as the stock biomass grows due to stocking. At some point an equilibrium would be reached and the sequence would oscillate between the same two levels of catch. However, the use of this design to estimate the effectiveness of stocking by comparing catches between years with and without stocking would underestimate the effectiveness of stocking at this level of fishing mortality, since the catches do not return to their prestocking level between years of stocking.



## Figure 5

Simulation of yield with F = 1.0 when stocking occurs on even numbered years. Parameters of the Ricker surplus production model with stocking are: A = 1.2, B = 0.007,  $a(H_i)^b = 20t$ .

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# Some Aspects of Offshore Spat Collection of Japanese Scallop

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## ABSTRACT

The recent information on offshore spat collection of Japanese scallop, *Patinopecten (Mizuhopecten) yessoensis* JAY, is briefly outlined. Scallop mariculture in Japan has developed rapidly due to technical advances in the methods for spat collection and intermediate culture made in the mid-1960s. The spat collected offshore is indispensable to Japanese scallop mariculture that has evolved since the mid-1970s. Nevertheless, offshore spat collection is frequently unsuccessful because of the intricate fluctuations in open sea conditions. Therefore, some research has been performed in recent years on larval monitoring. The results to date have shown that the scallop veliger larvae are distributed at comparatively higher densities in particular waters (temperature 7-8°C, salinity 32.0-32.5) along the coasts.

Scallop mariculture in Japan is a new and rapidly evolving mariculture industry, presently very important in the northern part of Japan (Ito 1988, 1989b). Its development is attributed to the success of mass production of scallop seeds. The seeds are produced from natural spat collection and intermediate culture in the sea. Although seed production first took place in embayment areas, it is now practiced in shallow waters of both embayment areas and the open sea. Off open coasts, spat collection is frequently unsuccessful; however, spat collected offshore have become indispensable to the Japanese scallop culture industry in recent years. Therefore, there is an urgent need to establish efficient methods for offshore spat collection. Some research has been done on this subject and has provided clues to the establishment of such methods.

In this report, the author will review the biology of Japanese scallop and trends in scallop production and offshore spat collection in Japan, and present results of recent offshore spat collection in Nemuro Straits, eastern Hokkaido.

# Biology of Japanese scallop \_\_\_\_

Japanese scallop (giant yezo scallop), *Patinopecten (Mizuhopecten) yessoensis* JAY, "Hotate-gai" in Japanese, is classified in the phylum Mollusca, class Bivalvia (Lamellibranchia or Pelecypoda), order Pteriomorphia, and family Pectinidae. This scallop is a cold-water species distributed in the subfrigid coastal areas of the north Pacific Ocean, the south Okhotsk Sea and the Japan Sea, along the coasts of the Kuril Islands, Sakhalin, Hokkaido, northern Honshu, Sikhota Alin, and northern Korea. The southern limit of the natural distribution in Japan is Toyama Bay on the Japan Sea coast and Tokyo Bay on the Pacific Ocean coast (Fig. 1).

The life cycle is as follows (Fig. 2): Demersal eggs fertilized in the sea after spawning. Fertilized eggs begin cleavage and reach the trochophore state at 4 days. The early veliger larva with a fully formed prodissoconch shell, called the D-shaped larva because of the straight hinge shell, is reached 5-7 days after fertilization. By 30-35 days, the umbones of the late veliger larva are fully grown and overhang the straight hinge. The pediveliger attaches to the substratum with byssal threads 40 days after fertilization. Immediately after attaching, rapid changes in shell morphology of the dissoconch (spat shell) and growth of internal organs take place, leading to the adult scallop form (Yamamoto 1964, Maru 1972).

After the attached spat have grown to about 1 cm shell length, they start to be released from the substratum and the spat inhabit the sea bottom. This scallop is gonochoristic in sexuality (Yamamoto 1943). Rarely, hermaphroditic individuals are found (Yamamoto 1964, Maru 1978b). Juveniles are lacking in a well-developed gonad and differentiate sexually at about 15 months. Adults are more than 2 years old



Figure 1 Main scallop mariculture areas in Japan and natural distribution in the northern sea.

(Wakui and Obara 1967; Maru 1976, 1978a; Osanai et al. 1980; Kawamata et al. 1981). The gonads grow from autumn to winter and become mature in spring. The eggs and sperm are released after maturation in the spring.

# Scallop production in Japan \_

Scallop mariculture developed rapidly due to technical advances in the successful methods of natural spat collection and intermediate culture in embayments in the mid-1960s. Scallop spat collection in Japan was first attempted in Saroma Lake in 1934 (Kinoshita 1935). After many experiments, a successful scallop spat collector was invented in 1964, primarily by Toyosaku Kudo, a fisherman in Mutsu Bay, Aomori Prefecture (Yamamoto et al. 1971, Tsubata 1982). The scallop collector is composed of a mesh bag filled with the substratum. The bag is made of a small or large synthetic fiber mesh, call "Japanese onion bag" in the small

mesh size and "netlon net" in the larger. The bag filler is usually netlon net and waste fishing nets. The spat collected by these collector bags hung from longlines are then caged for seed production during a period of several months. This cage culture for seed production is called "intermediate culture." The intermediate culture seeds are used for hanging culture in exclusively designated sea areas and for sowing culture on prepared sea bottoms.

Scallop production in Japan remained at a low level of 5,000-20,000 metric tons for a quarter of a century, until 1970. After this, the hanging culture production increased, mainly in embayment areas such as Mutsu Bay in Aomori Prefecture, and Funka Bay and Saroma Lake in Hokkaido (Fig. 3). After 1975, the sowing culture production increased rapidly, mainly in the coastal regions of Soya and Abashiri in north Hokkaido, facing the Okhotsk Sea. In 1982, the production of hanging culture amounted to 77,000 tons, and the production from sowing culture and fishing on wild stocks



### Figure 2

Life cycle of the scallop Patinopecten (Mizuhopecten) yessoensis (Jay), with notes on culture methods. (Modified from Yamamoto 1964; Maru 1972, 1976, 1978a,b; Kawamata et al. 1981.)

reached a little short of 100,000 tons, for a total production of 176,000 tons.

The scallop takes first place, by value, in molluscan shellfish production in Japan, with a three-fold increase in production and a four-fold increase in value over the last 10 years. The value in 1982 amounted to 42 thousand million yen. At present, sowing culture and wild production account for 56% of the total, and sowing culture keeps the scallop industry prosperous. The rapid development of sowing culture is attributed to the mass production of scallop seeds. The number of seeds sown around Hokkaido amounted to 1,500 million shells in 1982, a three-fold increase in the last 10 years (Fig. 4). Most of the seeds are sown in the coastal retions of northern and eastern Hokkaido in this order: Abashiri (990 million shells, 66%), Nemuro (200 million shells, 13%), and Soya (180 million shells, 12%). The seeds for sowing culture are produced in the Rumoi, Soya, and Nemuro regions, and depend mainly on offshore spat collection. These seeds, collected offshore, are mainly used for sowing culture. Therefore, offshore spat collection is equally indispensable as collection in embayments for sowing culture.



Recent trends in Japanese scallop production.



Figure 4 Recent trends in yearly seed input for scallop sowing around Hokkaido.

# Offshore spat collection .

Offshore spat collection off open coasts has been done commercially since the mid-1970s (Maru and Nakagawa 1979, Shiogaki et al. 1980). The spat collected offshore have accounted for half the seed supply for sowing culture since 1980 (Fig. 5). The offshore areas for spat collection operations are distributed along the coasts of the northern Japan Sea, the Okhotsk Sea, Nemuro Straits off Hokkaido, an Tsugaru Straits and the Pacific Ocean off Aomori Prefecture. Most of the offshore spat are collected around Hokkaido.

Environmental conditions of the open coast during spat collection fluctuate intricately because of seasonal changes in the water masses from spring to summer (Fig. 6,7). The Tsushima and Soya warm currents influence the waters around Hokkaido to raise the temperature at this time (Komaki 1975, Fujii and Sato 1977). Spat collection occurs in the coastal areas directly influenced by coastal waters, and indirectly influenced by oceanic waters.

The breeding season of scallops along the open coasts around Hokkaido extends from April to July. The scallop is induced to spawn by factors controlled by the animal itself and by environmental conditions. The gonad index (gonad weight  $\times$  100/soft body weight) and the water temperature are the important factors related to spawning, and are used as a precursor to monitor the planktonic larvae. The gonad index increases rapidly, reaches a maximum value in spring before breeding, and then rapidly decreases to a minimum in summer after breeding. Changes in the index are monitored and the breeding time is estimated. Increases in water temperature progress in the following order: Japan Sea coast, Okhotsk Sea coast, and the Nemuro Straits coast (Fig. 8). Likewise, scallop breeding along the open coasts around Hokkaido generally begins first on the Japan Sea coast, second on the Okhotsk Sea coast, and third in the Nemuro Straits (Fig. 9). Local differences in breeding times are well explained by differences in water temperatures. Coastal surfacewater temperatures during the breeding period range from 8 to 12°C; however, bottom temperatures are also partially involved. The coastal surfacewater temperature is 2-3°C higher than the bottom temperature. Thus, the scallops probably breed in 5-10°C temperatures, but accurate measurements have not yet been obtained.







Figure 5 Recent trends in number of scallop seed marketed.

The scallop larvae are monitored with plankton analysis for spatfall prediction. In embayments, the densities of the larvae usually range from 100 to 10,000 individuals/m<sup>3</sup> seawater (Maru 1985). Nevertheless, the offshore larvae are very few and their densities fall to lower levels, 10% to 1% of those in embayments (Fig. 10). As a result, industrial methods for offshore spat collection are at present not established, and collection is frequently unsuccessful. Moreover, data realted to this are scarce. Therefore, research has been carried out in offshore areas for spat collecting, and following are the results of our research in Nemuro Straits.

# Research in Nemuro Straits .

Industrial spat collection has been attempted since 1977 in Nemuro Straits (Fig. 11), east Hokkaido, near the southern Kuriles, although it was unsuccessful during the period 1977-81. In 1982, a research project for this region was begun by this author and co-workers of a special team organized to concentrate systematically on creative concepts of scallop mariculture (Ito 1989a). The research area extended along 350 km of coastline from the Shiretoko Peninsula facing the Okhotsk Sea to the Nemuro Peninsula facing the Pacific Ocean. Water depths investigated range from a few to 1000 meters, since the northern region becomes rapidly deeper than the southern. The vertical search range extended to depths of 70 m and to plankton living at less than 20-m depths. The biological and environmental research took place aboard four vessels at half-week or one-week intervals for 4 months, from April to July.





## Figure 7

Seasonal water masses around Hokkaido. A, Tsushima warm current; B, Tsugaru warm current; C, Soya warm current; D, northward current of the Kuroshio; E, coastal branch off the Oyashio (Kuril cold current); F, offshore branch off the Oyashio (Kuril cold current); G, east Sakhalin cold current; H, Liman cold current; I, west Sakhalin coastal water; J, cold water west off Tsugaru Straits; K, cold water west off Musashi-tai; L, inter-cool water; M, mixed water area of circulating current; N, floating ice area; O, coastal water area. (After Komaki 1975, Fujii and Sato 1977.)

## Figure 8

Surface temperature changes of coastal waters relative to locality around Hokkaido, 1982.







Figure 10 Changes in numbers of scallop veligers and attached spats on the coasts of Okhotsk Sea and Nemuro Straits off Hokkaido, 1982.



Figure 11 Location of Nemuro Straits.

In Nemuro Straits, the spawning period varied with the location of the scallop habitat. For 1982 it was estimated to be (Fig. 12) early-June to early-July at Rausu (northern region), mid-June at Shibetsu (middle region), and late-May at Bekkai (southern region). In short, the scallop spawned earlier in souther than in northern areas. This is well explained by the time lag in temperature increase. Bottom temperatures at spawning are similar at different localities, with a range of 5-7°C. Further, the scallop is induced to spawn by an abrupt rise in temperature. Spawning was observed in 1983 after a slight temperature increase, as little as 1°C, at the bottom, as a result of rough weather (Figs. 13, 14).

The distribution of planktonic larvae shows fast-moving kaleidoscopic changes, with time (Figs. 15, 16). Environmental conditions of the open coast also fluctuate intricately and are not easily forecast. However, it appears possible to draw conclusions from the phenomena observed. The larval distribution is not regulated simply by temperature and salinity, but seems to have some relation to water masses in the coastal areas. The waters with high densities of larvae have salinities of 32.0-32.5 and temperatures of 7-8°C (Fig. 17). Consequently, we have concluded that the larvae are distributed with comparatively high densities in particular water masses of the coastal waters.



Figure 12 Changes in gonad index and water temperature in Nemuro Straits, 1982.

The results mentioned above are preliminary, and more detailed research will be undertaken. However, industrial spat collection in the Nemuro Straits has been successful since 1982, thanks in part to the results of this research project.

At present, offshore spat collection is indispensable to scallop production in Japan. Some research has been performed to establish efficient methods for offshore scallop spat collection, and some information has been obtained on scallop spawning habits, larval distribution, and environmental conditions. In some regions, industrial spat collection is presently successful after the results of our project in the Nemuro Straits; however, successful methods for spat collecting in other regions need to be established.

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Figure 13 Changes in gonad index relative to sea conditions off Shibetsu, 1983.



Figure 14 Horizontal changes in bottom temperatures of adult scallop habitat off Shibetsu, 1983.



Figure 15 Weekly changes in density of scallop larvae in Nemuro Straits, 1982.



Figure 16 Daily changes in density of scallop larvae off Shibetsu, 1982.

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Figure 1/ Relationships among larval density, water temperature, and salinity in Nemuro Straits, 1982.

# Enhancement of Molluscan Shellfish in Washington State

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## ABSTRACT

The Washington Department of Fisheries has undertaken augmentation of natural stocks of three clam species. We are applying new enhancement methods in an optimal environment for molluscan shellfish to meet increasing seafood demands. The approach taken in each case has been to carefully evaluate both biological and economic aspects of enhancement and to select the method that best fits the particular circumstances. The work carried out demonstrates that a variety of techniques are available (hatchery seeding, transplantation of natural seed, and habitat improvement). However, it also clearly demonstrates that the more comprehensive the initial assessments can be, the greater the chances of success.

<sup>1</sup>Deceased July 1989.

The state of Washington has about 2,700 miles of marine shoreline and major stocks of molluscan shellfish yielding over 30 million pounds annually. Between 1950 and 1985, the State's population increased from 2.3 to 4.3 million people. At the same time, the demand for shellfish increased by a factor of about four due to increasing recreational and commercial use. At present, demand far exceeds the supply. To fill the need, effort has been directed to increasing stocks of shellfish, and this report describes enhancement methods being employed by the Washington State Department of Fisheries for three different clam fisheries.

Geoducks are large (up to 10 pounds) clams found primarily subtidally, but also low on intertidal beaches. This clam has long been popular on intertidal sport beaches, and is harvested commercially (up to 5 million pounds per year) by divers. The primary factor limiting geoduck harvest is its very slow natural recruitment rate, although the clams grow to commercial size rapidly. This creates an opportunity for enhancement through artificial seeding of the clam beds, and we are developing a hatchery system initially aimed at doubling current harvest levels.

Razor clams are a very popular sport clam taken along 60 miles of intertidal ocean beach. Harvest is by hand-digging at low tide. Digging pressure has more than doubled during the past 35 years, while clam setting has markedly decreased on about one-third of clam beds. One method used to increase the abundance of clams is to transplant naturally produced razor clam seed from subtidal areas of high abundance to the low-abundance areas on intertidal beaches.

Hardshell clams are highly prized by sport and commercial users. Clam abundance remains generally good, but demand far outdistances the supply. One of the major limitations on stocks is lack of suitable habitat for larval settlement and growth. These clams require a sand-mudgravel mix, and will not grow on mud or sand flats. The Washington Department of Fisheries and the industry are developing techniques for creating new clam beaches by spreading a layer of gravel 4-8 inches thick on non-clam producing beaches. High success occurs when beaches are carefully selected.

# Geoducks \_\_

During the summer of 1967, the Washington Department of Fisheries began surveying the shallow subtidal region of Puget Sound to determine the extent of the geoduck resource. These surveys, conducted by SCUBA divers, have continued to the present time. From the beginning of the surveys, large numbers of geoducks *Panope abrupta*, formerly *generosa*, were found, justifying harvest of this unexploited resource. In 1969, the Washington State Legislature provided for a commercial geoduck fishery beginning in 1970. Current law limits the fishery to divers using hand-held gear and to waters greater than 18 feet deep and further than 200 yards from the mean high-tide line.

# **Distribution and abundance**

Geoduck clams are found in North America from Alaska to California, with the population center in Puget Sound and British Columbia. Large populations of geoducks have also recently been reported in Japan. Survey results for geoducks in Puget Sound are as follows:

		Geoducks
	Acres	(million pounds)
Observed geoduck beds	33,799	
Major and commercial beds	19,545	280
Commercial beds	8,378	165
Annual harvest from		
commercial beds	200-300	5

# Life history

Geoducks are the largest-known burrowing clam in the world. In Puget Sound adult clams weigh an average 1.9 pounds, and may reach 10 pounds. A geoduck begins life in the spring, when spawning adults release millions of gametes into the water. For 3-5 weeks, larval clams drift with the currents and may be transported far from the parental bed. They soon lose their ability to swim, settle to the bottom, and burrow into the substrate, usually down to 2-3 feet, as they grow. Burrowing ceases as clams reach adult size.

The major factor limiting geoduck production is the very slow rate at which new clams are recruited into the population. Although the clams grow rapidly, it takes 15-60 years for a harvest population to be replaced naturally.

Geoducks are commercially harvested from subtidal beds by divers using hand-held water jets. The water jet used to harvest the clams is a short pipe ( $\sim$ 18 inches long) with 5/8-inch diameter tip at the digging end, and a shut-off valve on the other. Geoducks are harvested individually. The animal is located by its "show" (neck extended out of the substrate), or by feeling for depressions in the substrate left when the neck is withdrawn. The nozzle, placed next to the show, liquifies the substrate immediately around the clam, allowing the geoduck to be pulled out.

An experienced diver harvesting a good bed can dig a geoduck in 15-30 seconds, and may harvest up to 2,000 pounds per day under ideal conditions. Following is a summary of geoduck harvests in Puget Sound, 1970-85:

Year	Pounds
1970	82,236
1971	610,250
1972	493,140
1973	463,994
1974	803,358
1975	2,372,271
1976	5,365,898
1977	8,646,746
1978	7,089,656
1979	5,228,215

1980	3,910,192
1981	4,290,127
1982	5,303,081
1983	3,523,450
1984	4,421,265
1985	4,109,000

## Enhancement

A geoduck hatchery and juvenile grow-out facility is operating at the Point Whitney Shellfish Laboratory, Washington Department of Fisheries. The hatchery and its operation are financed through the Washington Department of Natural Resources from sales of geoduck harvest rights to commercial fishermen. The primary goal of the hatchery system is to restock harvested subtidal geoduck beds with cultured seed. Artificial seeding, if successful, could greatly reduce the time-interval between crops from individual beds. The first large-scale plantings were in 1985, when 250,000 seed were planted, and in 1986 when 1.6 million seed were planted. The long-range goal is to increase production in the hatchery until 30 million seed/year are available for planting to support an additional 5-million-pound harvest each year.

Hatchery Young clams (6-12 years old) are preferred for brood stock because of better quality eggs. Spawners are brought into the hatchery from December to July to coincide with the natural spawning season. Spawning is accomplished by stimulation with high-density algae. The larvae are normally ready to metamorphose 20-21 days after fertilization.

During the first 10 days, the larvae are fed a combination of *Chaetocerus calcitrans* and Tahitian *Isochrysis*. After collection on a 120- $\mu$  screen, they are fed a mixture of 70% *Thalassiosira pseudonana*, 20% Tahitian *Isochrysis*, and 10% *Dunaliella tertiolecta*.

**Field planting** The commercially harvested beds being replanted with hatchery seed are all subtidal, so the standard method of intertidal clam reseeding furing a low tide cannot be used. Two basic methods of subtidal seeding are: 1) Hand planting by divers (good only for small experimental plots), or 2) surface planting from boats, allowing the seed to fall to the bottom where they attach and burrow.

We have developed a geoduck seeder mounted on the back of a 28-foot boat. The seed is placed in a cone-shaped tank with seawater injected at the bottom. Eight siphons carry the suspended seed in equal amounts, via plastic hoses, to the eight distribution nozzles, each 4 feet apart. Seeding is accomplished by running the boat over the area to be seeded in long transects. Each pass covers a strip about 40 feet wide. Aliquots of seed are dumped into a cone tank as the boat proceeds down the transect line. By varying the number of aliquots per unit of time and the speed of the vessel, the density of seed reaching the bottom can be controlled. We normally plant between 10 and 20 seed/m<sup>2</sup>. The water currents and water depth affect lateral drift of the seed as it settles through the water column. The settling speed of the seed is directly related to seed size. Clams 8 mm in length settle through the water at a rate of 7 cm/ second.

Predation during seeding has been a major concern. However, significant loss of the seed as it passes through the water column has not occurred, even though various types of potential predators have been present during seeding. After landing on the bottom, the seed must burrow rapidly to avoid surface-feeding benthic predators such as flounders, soles, crabs, snails, and starfish. Burial time is inversely related to seed size. Seed 1-2 mm in size will become burried in 4-5 minutes; 10-mm seed requires up to 30 minutes for burial. During the first 2 years of life after planting, the seed is susceptible to such predators as starfish, crabs, and moonsnails, that can dig into the substrate and attach the juvenile clams. After 2 years, the clams are normally buried deeply enough in the substrate to be free of predation.

Seed survival after 2 years in small experimental plots has varied from 0 to 40%, with most experiments averaging between 2 and 5%. Predation is thought to be the primary reason for losses, although predator exclosures, such as screens and cages, have not increased survival. Seed size, substrate type, particle size, and compaction appear to be important to survival. Seed of 3-8 mm shell length at planting have never shown survival greater than 5%. Of seed averaging 13.5 mm shell length, 40% survived in one experiment in a compact muddy area; but only 1.35% survived in a soft sandy area.

Seed growth after planting varies according to the area planted, but can be very rapid. Planted seed can reach 2 pounds in 4 to 5 years.

## Razor clams.

The Pacific razor clam *Siliqua patula* inhabits Washington's open, wave-swept coastal beaches from the mouth of the Columbia River north to the Quinault River, and on small isolated beaches north to Cape Flattery. It supports an intensive recreational fishery on the Washington coast. Historical annual harvest effort has been about 700,000 digger trips, yielding approximately 1.3 million pounds of clams.

## Life history

The Pacific razor clam is found in North America from central California to Alaska, with the largest population available for recreational harvest occurring in Washington. Local razor clams may live 5-8 years; however, most are harvested by age-3. Reproductive maturity occurs by age-2, and spawning usually takes place in late spring. Larval survival and distribution are dependent on both favorable ocean currents and weather conditions.

Settlement of juvenile clams into the substrate occurs in very large numbers subtidally. In this comparatively stable

environment, they grow while subjected to tremendous natural mortality. They also move, either voluntarily or involuntarily, toward the beach. Some of the survivors are deposited on to the unstable high-energy intertidal beach as 5-15 mm juveniles. Within a year, the survivors are 2-2.5 inches in length and are recruited into the fishery the next year as 4-4.5 inch clams.

During the past 35 years, intertidal razor clam stocks have steadily declined due to intense harvest, inconsistent natural reproduction and reduced setting, and recent disease mortalities. In 1979 the Washington State Legislature directed that new efforts be undertaken to offset this decline, including enhancement of clam stocks to increase the number of razor clams available. One method being attempted is to seed intertidal areas of depressed natural clam abundance with large numbers of juveniles obtained from subtidal areas.

## Enhancement

The large populations of juvenile razor clams on recreationally inaccessible subtidal beds provide a potential source of seed for replanting intertidal sport beaches. Initial work involved developing gear and techniques to harvest the seed and transfer it to intertidal beaches, and then surveying the nearshore area for major beds of seed clams.

To harvest the seed clams, a small hydraulic-airlift harvester was developed. The clams are separated from the sand substrate by water jets and then lifted to the surface by an airlift. Harvest is from a 34-foot boat in shallow waters 10-40 feet deep during calm seas. The seed harvested is 5-15 mm in length.

In late July 1985, 21 tows were made in 6 days of harvesting. A total of about 9000 juvenile razor clams were harvested. Six tows on 6 August yielded over 6.3 million juveniles while the next day, 7 August, eight tows produced over 15.5 million juveniles. These high recoveries demonstrated the potential feasibility of the harvest method.

Subsequently, a special survey was undertaken to determine the extent of this available resource. Juvenile clam densities were found to be highest in the vicinity of the northern beaches. Between 6 August and 10 October, 84 tows yielded over 127 million juvenile razor clams. Of these, 93 million were transplanted to the beaches south of Grays Harbor, Twin Harbors, and Long Beach. Based on these surveys, it was conservatively estimated that there were approximately 28 billion juvenile clams present in the 5-km<sup>2</sup> subtidal area near Iron Springs where maximum densities exceeded 13,000/m<sup>2</sup>. Later harvests in early October produced over 19 million juvenile clams per day which averaged 5 mm (range 1-15 mm).

At the end of each day's harvest, the seed clams are taken ashore and manually planted on the beach at low tide. Several experimental planting methods were tried. The most successful was to place the seed in large buckets and pour the contents on the sand ahead of an advancing wave. Although the seed clams dig into the sand within minutes, planting is best done at night on an incoming tide to minimize dessication and predation. Even with this manual method, large plantings can be accomplished in a short time-period.

The harvest method is clearly successful and cost effective. The unknowns are:

1 What is the survival of the planted clams? Studies are underway to assess survival. Early indications are that adequate survival occurred.

2 Will subtidal sets occur with sufficient regularity to make this a useful technique? If seed is available at least once in 3 years, it will be viable. If seed is available only once in 10 years, it would be of limited value. In 1988, nearly 3.3 million juvenile clams were again harvested from the area near Iron Springs. These clams were also transplanted to the beaches south of Grays Harbor. Therefore, preliminary indications are that subtidal seed will be available at least once every 3 years.

# Hardshell clams \_

Sport utilization of hardshell (manila and native littleneck) clams has increased from about 250,000 user trips in 1950 to over 1 million in 1985. Commercial production has more than doubled. Total harvest currently is about 4.8 million pounds per year. Since one major limitation to production of these highly valued clams is lack of the needed sand-mud-gravel substrate for settlement and survival of clam larvae, there is a major focus on modifying new beach substrate to increase production. Starting in the 1960s, the industry, and more recently the Washington Department of Fisheries, have embarked upon beach enhancement by spreading 1-8 inches of gravel over previously unsuitable habitat to create new clam ground.

The gravel used is a mixture of rock measuring 1/4-inch up to a maximum of 3 inches in diameter. Normally, gravel is hauled-in by barge, dumped at high tide, and then spread mechanically at low tide. It normally takes a minimum of 3 years after placement before clam production begins.

Major factors in successful clam production include:

1 Selection of areas where good populations of free-swimming clam larvae are present.

2 Selection of areas where the gravel will stay effectively in place for at least 20 years. Areas of excessive storm (wave) exposure and of heavy silt deposition or major longshore current should be avoided.

3 Avoidance of areas with heavy organic loading in the existing substrate. Placement of gravel in these conditions usually results in production of hydrogen sulfide and will foul the ground for 2-4 years. It is also wise to avoid areas of dense eelgrass or seaweed. At the very least, graveling should be done when stands of grass are at a seasonal minimun. Freshwater run-off can result in siltation of gravel plots when combined with poor upland management.

4 Avoidance of areas with major abundances of known clam predators.

Cost of graveling is variable but is estimated to be about \$12,000 per acre. When in production, each acre should have an annual yield of 15,000 pounds of clams.

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The Role of

Aquaculture in

the Restoration

# ABSTRACT

The natural populations of most commercially important species of bivalve mollusks continue to decline in North America. As these stocks decline and their commercial and recreational values increase, aquaculture becomes an appreciably more attractive alternative for the restoration and/or enhancement of molluscan fisheries populations. The historical uses of aquaculture to initiate, and eventually supplement, the Pacific oyster fishery in the northwestern United States and British Columbia provide a good model for the development of fisheries management through the intercession of aquaculture. This paper reviews two approaches to the incorporation of aquaculture in the management of restoration or enhancement of molluscan fishery stocks: (1) Use of hatcheries as management tools, and (2) the role of genetics in mollusk fishery enhancement. Both approaches have significant potential for mollusk fisheries, but both are prone to an eventuality which can include long-term detrimental impacts on fishery populations. The inevitable conclusion is that, provided we learn from previous mistakes of the salmonid fisheries, both hatchery production and genetic manipulation of resource populations can provide significant relief to the molluscan fisheries of North America.

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Decreases in shellfish stocks, whether resulting from overfishing, parasites, predators and disease, or unusual environmental perturbations, often prompt management agencies to encourage or implement stock enhancement programs. These programs have as an ultimate goal either the rebuilding of depleted fishery stocks, or the augmentation of natural stocks to support an artificially high maximum sustainable yield. The shellfisheries of North America have not traditionally relied upon restoration or enhancement programs to augment wildstock populations. Fishery practices, such as the translocation of seed from natural beds to grow-out areas or the regular replanting of shellstock as cultch during periods when natural set is occurring, have long been part of the oyster industry (Beaven 1953, Tarver and Dugas 1973, Reisinger 1978, Dugas 1984). Similar activities, albeit on a more limited scale, have been practiced with other bivalves including the softshell clam, hard clam, and scallop (Turner 1951, Dow 1953, Mackenzie 1979).

Recently, the success of large commercial-scale mollusk aquaculture operations and the continued decline of wildstock resources have prompted an evaluation of the use of intensive aquaculture in the restoration and/or enhancement of wildstock fisheries. This paper will provide a survey of molluscan aquaculture technology applicable to restoration or enhancement programs, with particular attention to the use of hatcheries as a fishery management tool and the role of genetics in molluscan fishery enhancement. To place the problem in perspective, we will first address two case histories involving management practices influencing mollusk fisheries.

# A case history: Oyster culture in the Pacific Northwest \_\_\_\_\_

Aquaculture has played a significant role in the oyster industry of North America. Postset and seed of the American oyster, *Crassostrea virginica*, have been produced by hatchery and nursery systems for many years. This stock enhancement had been relatively small until recently when set, produced by commercial culture, has made a significant impact in certain parts of the United States. The oyster industry of Long Island epitomizes this impact. At present, one hatchery on Long Island provides nearly a third of the total oyster seed planted in western Long Island Sound. Hatchery-produced set are reared in trays suspended from rafts until they attain a size of 10-15 mm. The seed are then broadcast over prepared bottom for grow-out.

Although an appreciable dependence between hatchery/ nursery-produced seed and the oyster fishery of Long Island Sound has been demonstrated, development of the Pacific oyster, *Crassostrea gigas*, fishery and its continued existence depends on commercial aquaculture participation. For many years, no wildstock fishery for the Pacific oyster existed on the west coast of North America. All oysters were grown to market size from seed imported from Miyagi and Kumamoto prefectures in Japan. In the 1960s, *C. gigas* began appearing in significant natural spatfalls in Washington State (A.K. Sparks, Alaska Fish. Cent., Natl. Mar. Fish. Serv., NOAA, 7600 Sand Point Way N.E., Seattle, WA 98115-0070, pers. commun., Oct. 1986). These spat, along with those produced through commercial hatcheries, have supplied a greater and greater proportion of the seed used in the Pacific oyster grow-out industry (Chew 1979). The contribution of seed from hatchery intervention has become significant. One hatchery in Washington State produced 18 billion spat in 1986 (J. Donaldson, Coast Oyster Co., Quilcene, WA, pers. commun., Oct. 1986) and will supply seed to grow-out facilities from Quilcene Bay, Washington, to Humboldt Bay, California. The same hatchery has helped develop and standardize methods of transporting late-stage or "eved" ovster larvae for setting in other locales. This technique of "remote setting" has expanded the industry considerably by providing a low-cost, on-site seed producing capability to the growers of the Pacific Oyster.

# A case history: Spawner transplanting of the hard clam

Great South Bay on the southern shore of Long Island, New York, is the largest producer of the hard clam, Mercenaria mercenaria, in the world. This one fishery produces about \$20 million annually (Kassner and Malouf 1982) from landings that average about 400,000 bushels a year. The importance of this fishery is demonstrated by the number of management practices applied by state and local regulatory agencies. Among these are limitations on daily harvests, fishing gear, and minimum marketable sizes, as well as restoration programs involving the planting of seed clams and/or the transplanting of ripe spawners into the Bay during spawning seasons (Bricelj and Malouf 1981, McHugh 1981). The rationale behind the spawner transplants is based on the belief that highly fecund spawners introduced to the Bay from a more northern area would result in greater recruitment. It was thought an increase in recruitment would come about for two reasons: Spawners would add more larvae to the Bay milieu, and the spawning of the transplants would be asynchronous-spawning later than the native stock. The combined result of this introduction would thus be not only an increase in the total quantity of larvae available to the year-class but also an increase in the length of time that larvae would be in the Bay and exposed to favorable environmental conditions for settlement.

Recently, Kassner and Malouf (1982) performed a study evaluating the benefits of spawner transplants in Great South Bay. Their results, reported in Table 1, invalidate the assumption that the introduction of spawners serves to introduce larvae at a time when they would not otherwise be available from native stocks, at least during the year this study was performed.

If spawner transplanting does not provide larvae to the Great South Bay system when they would not otherwise be present, can it significantly supplement the natural set? Kassner and Malouf (1982) suggest that the contribution is

Table 1           Percentage of clams in spawning and spent stages of gametogenesis in native and transplanted populations of hard clams, <i>Mercenaria</i> <i>mercenaria</i> , in Great South Bay, New York (data from Kassner and Malouf 1982).						
Date	Native clams (%)		Spawner transplants (%)			
	Spawning	Spent	Spawning	Spent		
6/21/78	0	0	0	0		
6/25/78	18	0	35	20		
7/07/78	13	85	5	100		
7/20/78	0	100	0	100		
8/10/78	0	60	0	75		

negligible. They indicate that typical annual transplants involve about 500 to 1,000 bushels (18-36 m<sup>3</sup>) of chowder clams. Assuming there are about 250 clams/bu (7,000 clams/ m<sup>3</sup>), a typical operation would transplant 250,000 clams, one-half or 125,000 or which would be females. If it is also assumed that an average gamete release of Great South Bay female chowder clams is 10<sup>6</sup> eggs (Bricelj and Malouf 1981), then one could expect a maximum of  $7.5 \times 10^{11}$ larvae introduced to the system by the spawner transplants. Extrapolating from the data of McHugh (1981) who estimated egg production from natural clam populations in the eastern half of the Bay, we find that a total larval production for the entire Bay may well exceed  $11.0 \times 10^{15}$  per year. It would appear that the spawner transplants would contribute, at best, no more than 0.005% of the total annual larvae production in Great South Bay.

Although this analysis of spawner transplants in Great South Bay is less than encouraging, the concept of introducing spawners to provide larvae for areas devoid of clams is reasonable. Spawner transplants do have the capacity of providing seed to limited areas where larvae would not normally present.

# Hatcheries as a management tool \_

A paradigm of fishery management states that populationenhancement programs cannot consistently and predictably benefit fishery populations that are regulated by densityindependent factors. If, however, (1) a fishery population is regulated by density-dependent factors, (2) the environment is capable of sustaining introduced additions to this population, and (3) it is technically possible to produce large numbers of high quality seed on an economical basis, then hatcheries can be used to effectively intercede in fisheries restoration and management activities.

The success of finfish hatcheries in the augmentation of salmonid populations has stimulated interest in hatchery production as a management tool in molluscan fisheries (Malouf 1989). Augmentation of natural populations has a long history in the mollusk fisheries. Traditionally, seed transplanting programs, particularly for oysters, have been part of most fisheries on the eastern seaboard. In these fisheries, shellfish seed are taken from public or privately owned seed beds to areas where recruitment is low but growth and survival are excellent. The seed beds are restocked annually with shell or other cultch, normally at a time when shellfish are setting, to replenish the set and seed for future transplants.

In the northeastern United States hard clam seed is often planted by townships to augment natural clam populations and improve recreational and commercial fishing. As examples, townships in Massachusetts and New York typically purchase 4-6 mm seed clams from commercial hatcheries and utilize field nursery systems (rafts, cages, etc.) to grow the seed out to planting size (20-25 mm). These programs, however, are limited by the length of time available for effective field nursery culture and the availability of suitablesize seed in the early spring. Recently, at least one township in New York (Brookhaven) has begun buying young postset clams (0.5 mm) and using a land-based upflow nursery to produce the 5-mm seed for their field nursery system. Overall, the seed planting programs of the Northeaster United States are relatively small, and their value to the fisheries they were implemented to augment is questionable. A thorough evaluation of the planting programs has not been performed, and the factors influencing the potential success of such programs are not well understood.

In an analysis of two planting programs in New York and Massachusetts, Malouf (1989) concluded that clam seed planting programs of undetermined value should not be considered as benign at worst. These programs can result in the neglect of other management tools, the introduction of disease organisms or exotics, the possible reduction of genetic variability in natural stocks, and the possible degradation of growth rates and survival in natural populations by exceeding the carrying capacity of local environments. While it is presently difficult to justify the use of hatcheries to bolster declining commercial landings, it does appear that hatcheries can play a proper role in molluscan fishery management. Malouf (1989) stated that, as part of an integrated management program with realistic goals, hatcheries and other culture systems can be used to help establish or reestablish selfsustaining populations in localized areas, sustain recreational fisheries in intensively harvested areas, and provide a mechanism for allowing the genetic improvement in fishery stocks.

# Genetics in molluscan fisheries enhancement

Aside from the obvious benefits of improving growth and survival of molluscan stocks through genetic intervention, manipulation of the genetic architecture of molluscan populations can provide tools of significant value to fishery management. A good example is the recent development of triploid *Crassostrea gigas* and their incorporation into the Pacific oyster fishery of Washington State (Chaiton and Allen 1985). Although it appears that triploidy does not impart significantly faster growth in oyster seed (Stanley et al. 1981), it does interfere with gametogenesis, resulting in functionally sterile adults. This was an important result for the fishery, because it has traditionally suffered during periods when the resource was reproductively mature (ripe and spawning). An oyster with ripe gonads was unattractive for the half-shell trade, and subsequently the market and price were depressed in the spawning season. With the advent of triploid oysters and their characteristic lack of gonadal tissue, an opportunity developed to introduce a resource that would be attractive to the half-shell trade during the spawning season. The first commercial quantities of triploid *C. gigas* spat were produced in 1984, and the first harvest of market-size oysters has a high consumer acceptance and that the substitution of triploids for diploid *C. gigas* is an effective resource management tool (Jim Donaldson, Coast Oyster Co., Quilcene, WA, pers. commun., Oct. 1986).

Other examples of genetic manipulation of mollusks include the production of disease resistance in oyster populations and the genetic selection of shell markings for the production of identifiably distinct clams for restocking programs. The outbreak of MSX (Halplosporidium nelsoni) and subsequent decline of the oyster fishery in Delaware Bay in the 1960s prompted the establishment of a breeding program to produce disease-resistant oysters. The project has selected for MSX-resistant survival for several generations and has produced oysters for restocking that are many times more resistant than natural stocks (Ford and Haskin 1982). The general interest in restocking programs by management agencies has led to the production of seed with external characteristics allowing the easy identification or segregation of introduced populations. An obvious question applied to stocking programs is their real value in the contribution to the commercial or recreational harvest. Without a mechanism to identify the stocked populations, the actual contribution can only be speculated. In South Carolina, a project has been initiated to determine the benefits of restocking public shellfish grounds with hard clam, Mercenaria mercenaria. In order to determine the impact of such stocking programs and allow comparisons between introduced and native populasions, it was necessary to provide seed stock distinguishable from the native population. This was accomplished by crossing local clams with the "notata" variety of the same species. This variety has alternate bands of light and dark on the shell, making it easily differentiated from native stock. The shell markings have been demonstrated to be controlled by a single gene, or several closely linked genes, inherited as a Mendelian unit (Chanley 1961, Humphrey and Walker 1982). The seed generated through this cross has a very high percentage (80%) of notata coloration and will thus be segregatable from native wildstock which typically has less than 1% of the total population displaying notata markings.

A final example of genetic manipulation in molluscan fisheries is the recent work with heterozygosity in marine bivalve populations. Several reviews have brought considerable attention to the relationship between multiple-locus enzyme heterozygosity and growth rate (Koehn and Gaffney 1984, Gaffney and Scott 1985). Additional research has implcated improved survival and/or vigor with increasing heterozygosity (Rodhouse and Gaffney 1984). These data have stimulated the initiation of a breeding program to improve growth and survival in hatchery-reared populations of the hard clam, M. mercenaria, in South Carolina. This program incorporates three breeding schemes to produce improved stocks: Hybridization (with M. campechiensis), selected breeding, and outcrosses of inbred lines to induce increased enzymelocus heterozygosity. The lines produced through these outcrosses performed better than parental lines in overall growth over the first 12 months of culture but have not demonstrated increased heterozygosity. When compared with corresponding wild populations in regard to allele frequencies at seven polymorphic enzyme loci, the outcrossed lines showed evidence of genetic drift and loss of rare alleles. These crosses obviously resulted in the production of fast-growing lines that were genetically distinct from the parental stock (Dillon and Manzi 1987). Further analysis indicated that there were highly significant differences at individual enzyme loci in the largest and smallest clams from each cross (Dillon and Manzi 1988). These results would be consistent with the hypotheses that the alleles themselves were not effecting growth or were related to linkage disequilibrium. It appears instead that alleles are marking the entire parental genone, and that variation in growth rates of offspring from individual parents are masking a possible relationship with overall heterozygosity. This work is increasing the understanding of genetic variation in hatchery-reared seed and may allow more efficient and better-directed breeding programs for the production of molluscan seed stocks.

## Summary \_

Through the use of case histories and reviews of recent and ongoing research programs, this survey provided a brief recapitulation of the use of aquaculture, particularly hatcheries and genetic manipulation, in molluscan fisheries management. In summary, aquaculture-related fisheries management activities have significant potential for the mollusk fisheries of North America. Concentrating management programs in these activities can, however, result in neglect of other management tools and can lead to reduction of genetic variability in natural stocks and the possible degradation of growth rates and survival in local natural populations when introduced stocks exceed environmental carrying capacities. As part of an integrated fisheries management program, aquaculture can provide significant latitude in management options and can provide mechanisms for realistic stock improvements in fishery populations.

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# Application of LHRH-a Cholesterol Pellets to Maturation of Finfish: Milkfish

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# ABSTRACT

One step toward the goal of stock enchancement of a particular species is the control of its maturation in captivity. Many cultivated finfish species, however, do not complete the reproductive cycle in captivity. Hormone therapies have been used to overcome the physiological barriers that inhibit completion of the reproductive cycle. Acute or chronic hormone administration is often used to deliver various hormones or steroids to the fish. The slow and sustained release of LHRH to trigger the secretion of endogenous hormones is one hormone therapy attracting more attention.

The application of LHRH and its analogues and their potency for manipulating the reproductive activities of various fish, especially milkfish (*Chanos chanos*), is documented in this report. It has become essential in current aquaculture practice to control the reproductive cycle of cultivated fish in captivity. The traditional method of collecting fry from nature for aquaculture purposes is gradually being replaced by production of fry in the hatchery. Another objective of hatchery fry production is for release into the ocean for stock enhancement or for ocean ranching, as in the case of salmon and many species cultured in Japan. These goals depend on the successful maturation and spawning of adult fish in captivity, as well as on larval rearing. Unfortunately, these processes, particularly spawning, occur sporadically in most cultured species.

The purpose of induced spawning is to bring about the final stage of maturation in both males and females. In males, this stage includes spermiation and ejaculation. In females, it includes the migration and breakdown of the germinal vesicle, hydration, ovulation, and oviposition. Many researchers have reviewed the techniques currently used to induce spawning (Harvey and Hoar 1979, Lam 1982, Donaldson and Hunter 1983, Scott and Sumpter 1983, Lam 1985). Successful spawning can be achieved in most cases by a variety of methods including the use of hormones. After fertilized eggs are obtained, larvae are hatched and reared in a hatchery. The standard procedure for larval feeding begins with rotifers, brine shrimp, and/or copepods and is followed by a formulated feed. These procedures were reviewed by Kuronuma and Fukusho (1984) and Fukusho (1985).

Spawning can be induced and viable larvae produced only after the fish reach a certain stage of maturity. Some fish species, however, either do not complete or even begin maturation in captivity. Techniques for inducing maturation are still under development.

Induced maturation requires a more prolonged period (weeks to months) of hormone therapy than induced spawning. Hormone therapies are used to induce maturation by overcoming the physiological barriers posed by lack of necessary environmental stimuli. Environmental cues are known to play an important role in regulating reproduction and mediating the secretion of hormones which synchronize the activities of various organs into an orchestrated physiological and biochemical response. An effective hormone therapy must combine the most productive hormone formulation with the proper administration strategy. Among the available hormones and methods, LHRH, incorporated in a cholesterol pellet for implantation, has attracted increased attention (Donaldson and Hunter 1983, Crim 1985, Lam 1985).

In this report, we discuss the technologies for administering LHRH and document its potency for advancing and accelerating maturation and the reproductive cycle and for synchronizing spawning of various fish species. Finally, milkfish will be used to illustrate the potency of LHRH-a in inducing the maturation and spawning of important cultured fish. Table 1 Structure of LHRH.

pGlu-His-Trp-Ser-Tyr-Gly-Leu<sup>7</sup>-Arg<sup>8</sup>-Pro-Gly-NH<sub>2</sub> Mammalian LHRH

pGlu-His-Trp-Ser-Tyr-Gly-Trp<sup>7</sup>-Leu<sup>8</sup>-Pro-Gly-NH<sub>2</sub>

Salmon LHRH

# Luteinizing hormone-releasing hormone (LHRH)

The hypothalamus-pituitary-gonad axis is known to be the neuro-endocrine control route for fish reproduction (Donaldson 1973, Lam 1985). The hypothalamus controls the secretion of gonadotropin-releasing hormone (GnRH) which acts on the pituitary, causing it to release gonadotropin. Activities in the hypothalamus are controlled by environmental and/or hormonal factors, a process which usually takes a long time to initiate. Theoretically, direct administration of GnRH should shorten the time required to release gonadotropin. Research has been conducted to identify the structure of GnRH in fish (Sherwood et al. 1983, 1984). These researchers concluded that mullet, milkfish, trout, and salmon all contain chromatographically and immunologically identical peptides (Table 1). The structure differs from porcine (Matsuo et al. 1972) and ovine (Burgus et al. 1972) LHRH in two amino acids in positions 7 and 8 of the decapeptide (Table 1).

LHRH has since been synthesized in the laboratory, based on this proposed structure, and has proven effective in stimulating the release of gonadotropin and in inducing ovulation in mammals, chickens, and amphibians (see review by Lam et al. 1976).

Although LHRH was first proven to stimulate the secretion of gonadotropin in the common carp, Cyprinus carpio (Breton and Weil 1973), its potency is many times less than the synthetic nonapeptide LHRH (CTHAP 1977). Chinese scientists were able to induce ovulation in cultured carp with synthetic LHRH analogue. Several types of LHRH-a are available and have been tried on fish. These include des-Gly10[D-Ala6]-LHRH ethylamide; des-Gly10[D-Leu6]-LHRH ethylamide; des-Bly<sup>10</sup>[D-Ser(Bu<sup>t</sup>)<sup>6</sup>]-LHRH ethylamide; des-Gly<sup>10</sup>[D-Trp<sup>6</sup>]-LHRH ethylamide and des-Gly<sup>10</sup> [D-Phe<sup>6</sup>]-LHRH ethylamide. From an *in vitro* study conducted by Coy et al. (1975), luteinizing hormone (LH) and follicle-stimulating hormone (FSH)-releasing activities of the LHRH-a are ten times that of LHRH. The analogues also have a longer half-life due to a slow rate of enzyme degradation (Buckingham 1978). Marks and Stern (1974) also stated that des-Gly<sup>10</sup>[D-Ala<sup>6</sup>]-LHRH ethylamide is less readily broken down by brain enzymes than LHRH.

# Administration of LHRH \_

Modes of administration also affect the potency and half-life of LHRH in the fish. Crim (1985) discussed different administration methods, and other experiments were conducted to compare different application strategies (CTHAP 1977, Weil and Crim 1983, Kouril et al. 1983). LHRH/LHRH-a can be delivered to the fish at different body sites and using different vehicles. Injections can be made intracranially, intraperitoneally, and intrapericardially or intramuscularly. CAHEF (1975) indicated that effective dose of LHRH in most cultured carp species was lower when delivered by intracranial injection than with intraperitoneal of intramuscular injection. Only slightly better results were found with intracranial injection than with intraperitoneal injection for inducing ovulation in goldfish (Lam et al 1976). Kouril et al. (1986) conducted experiments with tench (Tinca tinca) and found that there was no difference in the number of spawned fish between intrapericardial and intramuscular injection of LHRH-a.

The vehicle used to deliver hormones to fish determines the rates of release and diffusion of the hormones and, consequently, the release and diffusion rates of gonadotropin. LHRH and its analogue have been administered to fish in either liquid or solid form. The liquid form consists of LHRH dissolved in 0.8% NaCl solution or in 40% propylene glycol. Administration of this form results in a rapid initial increase of the circulating hormone level but it is not sustained. Aida et al. (1978) prolonged the release of LHRH for a few days by using a viscous liquid vehicle. They prepared an emulsified solution of synthetic LHRH in Freund's adjuvant. Administration of the hormone in solid form sustains hormone release from weeks to months. Chronic LHRH administration devices have been prepared in the form of either a silicone rubber implant or cholesterol pellet. Both methods eliminate the need for frequent injections, thereby decreasing stress.

Preparation of LHRH silicone rubber implants has been described by Lotz and Syllwasschy (1979). Kent et al. (1980) described a cholesterol matrix pellet suitable for delivery of LHRH-a. This method was further modified by Lee et al. (1985, 1986c) to meet specified experimental needs. The basic composition of the LHRH-a cholesterol pellet is 95% cholesterol, 5% cocoa butter, and approximately 1% of the above total as LHRH-a. Following the procedures established by Lee et al. (1986b), each pellet weighs 20 mg, measures 2.4 mm in diameter and 5.0 mm in length, and contains 200  $\mu$ g LHRH-a. A bioassay study on this pellet was conducted on trout by Dr. L.W. Crim (Memorial Univ., Newfoundland, Canada). Pellets were implanted intraperitoneally (IP) or intramuscularly (IM) in rainbow trout. Blood from each fish was sampled on day 1 (the day of implantation) and again at 1, 2, and 4 weeks after implantation. Gonadotropin levels in blood serum showed a rapid elevation and remained at a higher level than in the control group for up to 4 weeks (Fig. 1).



Figure 1 Gonadotropin levels in trout after receiving a sham pellet or LHRH-a pellet.

# LHRH and the reproductive cycle in fish \_\_\_\_\_

Although LHRH did not elicit any change in prespawning landlocked salmon's pituitary GtH content (Weil and Crim 1983), LHRH and its analogue have proven effective in increasing plasma GtH levels in many other fish species (see review by Lam 1982, Donaldson and Hunter 1983). Elevation of the GtH level results in maturation and spawning of fish. Many researchers, therefore, began using LHRH-a to replace conventional HCG and fish pituitary. The response of fish to LHRH-a varies, however, according to the stage of gonadal development at which the fish receives the hormone (Crim et al. 1983a). In prespawning salmon, ovulation and spermiation were accelerated by LHRH-a treatment, but in sexually regressed male salmon, spermatogenesis was not induced by the same treatment. The LHRH-a treatment accelerated vitellogenic development during the rapid phase of gonadal recrudescence, but reduced the GSI value in male salmon. Crim and Glebe (1984) induced early spawning in 30% of the female Atlantic salmon given LHRH-a 45 days prior to the normal spawning season, and in 94% of the females treated 28 days before. LHRH-a cholesterol pellet treatment did not result in accelerated maturation or spawning in grey mullet when given approximately 60 days prior to the spawning season (Lee and Tamaru 1988). Ovulation in spring-spawning rainbow trout was advanced by 3 to 4 weeks, however, when fish were implanted about 2 months before the spawning season (Crim et al. 1983b). The spawning season in sea bass was advanced by 40 days using an LHRH-a injection under natural conditions (Barnabe and Barnabe-Quet 1985).

The potency of LHRH-a, when used to accelerate the maturation process, is inconsistent among species. LHRH-a has been successfully used to induce ovulation and spawning, however, in many fish species. These include: Acipenseridae (Doroshov and Lutes 1984); Anguillidae (Research Group of Eel Reproduction 1978); Cyprinidae (CTHAP 1977); Mugilidae (Lee et al. 1987); Plecoglossidae (Hirose and Ishida 1974); Serranidae (Barnabe and Barnabe-Quet 1985, Harvey et al. 1985); Siganidae (Harvey et al. 1985); and Soleidae (Ramos 1986). Spawning success was improved, however, by combining LHRH-a with other ovulatory agents such as: carp pituitary for mullet (Lee et al. 1987); 17  $\alpha$ -hydroxy-20 $\beta$ -dihydroprogesterone for carp (Breton et al. 1983), coho salmon and rainbow trout (Jalabert et al. 1978); and pimozide for goldfish, common carp, trout, catfish, and loach (see review by Lam 1985).

Pimozide potentiates the ovulatory effect of LHRH-a by blocking the action of dopamine which can mimic gonadotropin-releasing inhibitory factor (GnRIF). Pimozide can either be administered with LHRH-a or separately to the recipient fish. Billard et al. (1984) preferred to apply pimozide prior to LHRH. Sokolowska et al. (1984), however, concluded that the occurrence of ovulation in goldfish was high when pimozide was injected prior to or in conjunction with injections of LHRH-a. De Leeuw et al. (1985) indicated that the minimum effective dosage for inducing ovulation in African catfish could be as low as 5 mg pimozide combined with 0.05 mg LHRH-a per kg body weight. Lin et al. (1985) and Billard et al. (1984) have also conducted dose-response studies on pimozide and LHRH-a used to induce spawning in loach and brown trout.

Studies on the use of LHRH-a for fish reproduction have, thus far, concentrated on freshwater species. Recently, a research group at the Oceanic Institute in Hawaii has attempted to control the reproduction of milkfish, *Chanos chanos*, a euryhaline fish, by application of LHRH-a.

# LHRH-a and milkfish reproduction

# Maturation

The milkfish, an important food fish in Southeast Asia, especially in the Philippines, Taiwan, and Indonesia, rarely matures and spawns in captivity. Development of a reliable method for controlling maturation and spawning in milkfish has been investigated for a number of years (Lam 1984, Kuo 1985). The problems remain unsolved, however. Recent studies carried out by Lee et al. (1986a,b,d) indicate that LHRH-a has a positive effect on both maturation and spawning in milkfish.



Figure 2

Percentage of fish that reach maturity in response to different hormone therapies. Solid bar indicates females; blank bar indicates males. Fractions represent the number of mature fish to the total number of sexed fish. (Condensed from Lee et al. 1986b).

As mentioned, induction of the maturation process requires a prolonged period (weeks to months) during which circulating levels of the desired hormone must remain elevated. Among the varous methods of administration, the LHRH-a cholesterol pellet has accelerated the reproductive cycles of landlocked salmon (Crim et al. 1983a), rainbow trout (Crim et al. 1983b), and Atlantic salmon (Crim and Glebe 1984). In our 1985 milkfish maturation study, LHRH-a cholesterol pellets were applied alone or in conjunction with 17 $\alpha$ -methyltestosterone. The three hormone therapies used were: Cholesterol pellets containing 200  $\mu$ g of LHRH-a (LHRH-a pellet) or combinations of LHRH-a pellets plus silastic tubing containing either 250  $\mu$ g of dissolved 17 $\alpha$ -methyltestosterone (liquid MT capsule) or 10 mg crystal 17 $\alpha$ -methyltestosterone (crystal MT capsule).

Experimental groups of 20 milkfish each received one of these three therapies beginning in March, while a fourth control group received placebo implants. LHRH-a pellets were administered monthly; crystal MT capsules were administered once; and liquid MT capsules were administered twice, at the beginning of the experiment and three months later. The application of the LHRH-a pellet alone was less effective in inducing maturity in males but appeared to enhance the total number of females that reached maturity (Fig. 2). Mature females were found as early as April. The combination of LHRH-a and MT, in either crystal or liquid form, resulted in a significantly higher number of mature individuals by the month of July when compared with all of the other treatments (Fig. 2).



Figure 3 Maturation of male milkfish in control and treatment groups during 1986 season.

In the LHRH-a and liquid MT therapy, two mature females were also found in the month of April. The number of mature females increased steadily during the course of the experiment. By July, almost 90% of the individuals were found to be mature.

When LHRH-a was combined with MT in crystal form, a high percentage of running ripe males were found by June. A relatively low number of mature females was found in this particular hormone therapy. Overall, 65% of individuals undergoing this treatment matured by the end of July (after 4 months of treatment). Only one female from the control group reached maturity (possessed 0.7 mm oocytes) during the course of the experiment, attaining this state in July. Four males were found to possess milt as early as April. This number declined steadily until the month of July, when there was a dramatic increase. Therefore, the combination of LHRH-a pellet with liquid MT capsules appeared to enhance the maturation of milkfish.

In order to validate the effectiveness of LHRH-a pellets and liquid MT capsules, this combined hormone therapy was applied to 80 fish beginning in March 1986 (as Treatment 1) and in April (as Treatment 2). The 20 other fish in this study received placebo implants and served as controls. Maturation of males was not significantly enhanced by this hormone therapy (Fig. 3). By June, however, 90% of the females possessed eggs larger than 50  $\mu$ m compared with about 35% in the control group (Fig. 4). These results demonstrated that a combination of LHRH-a pellet and liquid MT capsule will enhance the maturation of female milkfish.



Figure 4 Maturation of female milkfish in control and treatment groups during 1986 season.

# Spawning

The formulation of a standardized method to induce milkfish to spawn has eluded investigators for over a decade (Lam 1984, Kuo 1985). Initially, spawning attempts involved administering piscine pituitary extracts (salmon or carp), plus HCG (Vanstone et al. 1977, Juario et at. 1979, Kuo et al. 1979, Liao et al. 1979). More recently, HCG has been used alone to bring about the final maturation of ova (Tseng and Hsiao 1979; Lin 1982, 1984). In all previous attempts, however, fertilized eggs were obtained by manual stripping of both females and ripe males for their gametes. This action usually resulted in the loss of broodstock, in only a single spawning per season, and in a low fertilization rate (0-60%). These factors clearly underscore the need for a more reliable method of inducing milkfish to spawn. This method should insure a higher fertilization rate, survival of spawners after spawning, and multiple spawnings.

In many cultured species, LHRH/LHRH-a has been used to replace traditional ovulating agents such as HCG or piscine pituitary (see review by Donaldson and Hunter 1983). We therefore evaluated the effectiveness of LHRH-a as a spawning agent for milkfish, when administered in either pellet implants or injections. Induced spawning was attempted when a female possessed an average egg diameter of 650  $\mu$ m or more. The maturity of males was assessed by exerting pressure on the abdomen and observing whether or not milt could be extruded. In each spawning attempt, LHRH-a was only administered once through either intramuscular pellet implantation or injection, in contrast to two or more injections in conventional spawning trials. A fixed dose of 250  $\mu g$  per fish was administered to all females and males receiving hormones. Control fish were either injected with normal saline or not treated at all. A total of 50 induced spawning attempts was conducted using either intramuscular pellet implatation (N=17) or injection (N=33). LHRH-a pellets were used April-July, and LHRH-a injection trials were



## Figure 5

Frequency of successful and unsuccessful induced spawnings of milkfish, attempted April-November 1985. Two strategies were employed in inducing final maturation and spawning: 1) LHRH-a cholesterol pellet implants, and 2) LHRH-a liquid injections. (From Lee et al. 1986b).



### Figure 6

Number of successful and unsuccessful induced spawns versus average egg diameters at which hormonal therapies were initiated. Results are presented for two different modes of administration (P=pellet, I=injection). (From Lee et al. 1986b).

conducted July-November (Fig. 5). Overall, a 53% spawning success rate was obtained using the pellet implant as a spawning agent. The fish consistently spawned about 48 hours after being implanted. Nineteen of 33 attempts using LHRH-a administered via an injection resulted in successful spawnings (58% success rate). In contrast to those induced with pellets, successful spawnings from fish injected with LHRH-a occurred within 20–26 hours after receiving their injections.

The number of successful spawnings is related to the average size of eggs at which the LHRH-a was administered (Fig. 6). The number of successful spawnings appears to increase with size of initial egg diameters, and 700–950  $\mu$ m is the optimal range. Successful spawnings occurred in fish that possessed single modal distribution of egg size in some fish that possessed bimodal distributions. In the latter case, the smaller clutch of eggs did not exceed 350  $\mu$ m in size. Spawning attempts in fish that possessed bimodal distribution of egg sizes, where the smaller clutch exceeded 350  $\mu$ m, did not succeed.

In the above experiment, the dosages of LHRH-a per kilogram body weight in spawning attempts were 41.3  $\mu$ g for pellet implantation and 58.7  $\mu$ g for liquid injection. Experiments are in progress to determine the minimum effective dosage for the spawning of milkfish.

In summary, LHRH-a is a potent hormone for controlling the reproduction activities of many finfish species. These activities include advancing and synchronizing spawning, increasing milt volume, and inducing spermiation. LHRH-a cholesterol pellets induce the release and sustain higher blood serum levels of GtH. The proper combination of LHRH-a cholesterol pellet with other hormones should control the maturation of most finfish species. This technology will benefit the enhancement of natural populations and the initiation of ocean ranching.

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# Trends in Oyster Cultivation on the West Coast of North America

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## ABSTRACT

Trends in oyster cultivation on the west coast of North America are briefly discussed with comments on species utilized, culture growout methods, remote setting of eyed larvae, and stock development through genetics and breeding. Although several species of oysters are utilized, the Pacific oyster (*Crassostrea* gigas) constitutes over 98% of oysters produced on the west coast.

Oysters are grown mainly on intertidal bed areas. Off-bottom culture, utilizing several techniques, is increasing but unlikely to expand quickly because of permit-application requirements related to the sociopolitical climate existing in most populated areas of the United States.

The concept of remote setting of eyed larvae has been shown to greatly enhance seed production of Pacific oysters on the west coast. Obtaining adequate Pacific oyster seed periodically from natural catches when available, backed up with a consistent annual catch from remote setting, makes seed production a concern of the past.

Stock development through genetics and breeding studies shows the potential for developing stocks resistant to diseases, as well as strains with desired traits. Breeding programs can be developed to produce high summer carbohydrate levels in oysters. Essential safeguards against inbreeding problems are discussed as they relate to Pacific oysters.

The method for producing the triploid oyster with greatly reduced gonadal development in the summer is discussed. These neutered oysters are expected to be in heavy demand for the summer fresh and/or half-shell trade when regular production is established. Over the past 25 years dramatic changes in commercial oyster operations have taken place on the west coast. The most significant changes are centered around the procurement of seed oysters and new innovations related to the development of desired stocks for cultivation. Further, although intertidal bed culture is still the main growing technique utilized by the oyster growers, we see an expansion of off-bottom cultivation.

The main oyster produced on the west coast at the turn of the century was the Olympia or native oyster (Ostrea *lurida*). With the decline of the native oyster industry, the eastern or American oyster (Crassostrea virginica) was introduced for cultivation, primarily in the state of Washington. Survival was not good for this species and ultimately the Japanese or Pacific oyster (Crassostrea gigas) was introduced and became the mainstay of the present west coast oyster industry. Aside from the Pacific oyster and, to a limited extent, the cultivation of the Olympia oyster in recent years, efforts have been made to utilize the Suminoe oyster (Crassostrea rivularis) (Breese and Malouf 1977) for cultivation primarily as a summer oyster. Also, efforts to produce the Kumamoto variety of C. gigas for hatchery production have met with moderate success. Later activities involved the growing of European oysters (Ostrea edulis) and hybridized Miyagi and Kumamoto varieties of C. gigas to produce what is called a gigamoto oyster. Although smaller, the gigamoto will grow a deeper shell and provide a good shape and taste for the half-shell market. Chew (1984) discussed Pacific ovster production trends for Washington, California, Oregon, and British Columbia and noted that Washington is still the major producer for the west coast. In fact, Washington's annual production has risen from over 6 million lbs (2722 MT) of meat in 1985 to 10 million lbs (4537 MT) in 1989. Recent estimates of 1989 production in California are above 1 million lbs (454 MT), and British Columbia and Oregon are about 0.5 million lbs (227 MT) each. Alaska has also begun production of Pacific oysters but at a much lower level.

## Culture grow-out methods -

Extensive culture with Pacific oyster seed placed on intertidal beds for growout remains the major west coast production method, probably accounting for more than 90%, with the rest produced by off-bottom culture.

Further, *Crassostrea gigas* probably constitutes more than 98% of oysters produced on the west coast of the United States and British Columbia. Figure 1 provides a map of the major areas of natural seed production and growout of commercial stocks from British Columbia south to California. Although not shown, southeastern Alaska is also beginning to produce Pacific oysters, primarily for the half-shell market.



Figure 1 General area of Pacific oyster production.

During the past 20 years, several methods of off-bottom cultivation for Pacific oysters and other species have been initiated. Each are briefly described below.

Long line culture involves the establishment of a system on the intertidal beds where shell cultch material with seed is strung between poles on heavy braided polypropylene rope. This is a growing-culture operation at present and occurs in Grays Harbor and Willapa Bay, Washington. Floating longline culture with buoys has been used in northern Puget Sound on a limited scale for hanging lantern and pearl nets for growing oysters. **Stake culture** occurs in several areas. Wire or wood stakes with seed-bearing cultch material attached to the top are placed in the lower intertidal zone.

**Rack culture** is one of the more popular type of off-bottom cultivation. Racks are built in the intertidal zone and shell strings are hung for production of oysters. This type of culture is found in all four California areas in Figure 1 and is increasing in Willapa Bay and Grays Harbor, Washington. Alaska and British Columbia are also expanding this culture technique.

Also, French plastic pipe collectors with seed can be used in a type of rack culture in British Columbia and Washington. Double rows of line are staked on the intertidal zone and the plastic pipes are laid across and fastened to the line.

**Raft or floating culture** The commercial use of rafts for hanging oyster strings continuously in the water column was initiated more than 20 years ago in southern Puget Sound, but has not expanded for a variety of reasons. Aside from minimal biological concerns, there are problems with securing permits for such applications through governmental agencies. Generally, these problems relate to multiple-use conflicts, aesthetics, navigation, and environmental concerns, all affecting the premit process.

Lantern and/or pearl nets Several operations in Washington and British Columbia utilize these types of hanging nets on long lines or rafts to grow oysters for the half-shell trade.

**Trays** Plastic netting is used in many areas along the west coast for the production of single Pacific oysters and other species for the commercial half-shell market. The method of using Vexar plastic netting was adapted from the French technique whereby sheets of material are folded over and sewn to make a basket. These baskets or trays are laid on a special metal or wood frame (rack) on the intertidal bed. One of the major operations using this technique is in Willapa Bay, Washington, and several other areas along the Pacific coast are running tests to determine their feasibility.

# Remote setting for seed \_

In recent years Pacific coast hatcheries have become very important to the oyster farmers. For many years, consistent supply of seed oysters to maintain annual production was dependent upon supplies from Japan, especially during the post-World War II years prior to the 1970s. As Pacific oyster seed shipments from Japan declined, natural catches of seed became available from some locations along the west coast (Pendrell Sound, British Columbia, and Hood Canal and Willapa Bay, Washington). Figure 2 summarizes in part the seed production for the state of Washington during the years 1947–85. As shown in this figure, most of the Pacific oyster seed from Japan remained in the state of Washington. Although natural-caught seed may be available in Hood Canal, it is not a dependable source of seed every year. For example, the two bays in north Hood Canal (Quilcene and Dabob


Figure 2 Production of seed oysters in Washington compared with Japanese seed shipments to the U.S. Pacific coast, 1947–85.

Bay) as a general rule will produce commercial quantities of seed only six or seven years out of ten, a difficult situation for the oysterman who depends on this seed source.

A new culture technique has been recently initiated on the west coast in which setting-size eyed Pacific oyster larvae can be purchased from private hatcheries by the oyster growers. The larvae can be kept alive for several days at 5°C in a cooler and sent great distances by air. Thus, the oyster grower can have a tank built on the farm and eyed larvae ordered for settlement. Although this concept, referred to as remote setting, has been tried experimentally for more than 20 years, it did not become an economical and accepted practice until 7-8 years ago. Presently two main hatcheries, one in Oregon and one in Washington, produce over ten billion eved larvae annually to supply the needs of the west coast growers who use remote setting to obtain seed. This process was well described by Jones and Jones (1983). As shown in Figure 2, there has been a dramatic increase of over 80,000 cases of seed produced from hatchery eyed larvae in 1985 alone, and over 100,000 cases annually since then.

In 1983 there were an estimated 18 oyster farmers building their own tanks to catch Pacific oyster seed from hatchery-produced eyed larvae. Recent estimates show that over 50 farmers from Alaska to California, including British Columbia, are using remote setting to securing seed for their oyster operations.

The cost of eyed larvae ranges from 8 to 12 cents/thousand depending on the time of year and the hatchery. Early research by Henderson (1983) has shown the importance of temperature and salinity for eyed larval settlement (Fig. 3). As demonstrated in Figure 3, remote setting tanks should be about 30°C and 30 ppt, respectively, for optimum results. Henderson also ran tests to determine the percent settlement after 0–12 days in 5°C storage (Fig. 4), and was able to show that the Pacific oyster eyed larvae should not be stored at  $5^{\circ}$ C beyond 8 days for best larval settlement or survival.



#### Figure 3

Cumulative mean percent larval *Crassostrea gigas* settlement at five temperature levels  $(15-35^{\circ}C)$  and at five salinities  $(15-35^{\circ}/\omega)$ . Points of intersection indicate factor interaction at the given coordinates. (From Henderson 1983)



Figure 4

Mean percent larval Crassostrea gigas settlement after 0-12 days in 5°C storage. Comparative mean spat survival after 90 days for each 48-hour storage interval is displayed above. Vertical bars indicate standard error of means. (From Henderson 1983)

This is basic background information, but the oyster farmer will need to determine the requirements of his own system to obtain best success.

Recent activities related to remote setting involves the use of the chemical L-3, 4-dehydroxyphenylalanine, commonly referred to as L-Dopa, which is also sold by one hatchery selling the eyed larvae. L-Dopa is used to facilitate larval settlement in the farmers' tanks. As a general rule, one can expect between 20% and 30% of the eyed larvae to metamorphose and settle successfully. Discussions with several oyster farmers reveal that a higher percentage is regularly achieved.

After the larvae have settled, it is possible to feed them for a day or two before they are taken from settling tanks to the outside environment. Hatcheries also sell a concentrated algal paste or algal slurry (Krantz et al. 1982). The algal cultures, usually made up of Tahitian *Isochrysis* or 3H (*Thalassiosira*), are grown in large tanks and passed through a mechanical centrifuge for concentration into a paste. Thus, the hatchery selling the eyed larvae can include sales of L-Dope and algal slurry to facilitate the remote setting of Pacific oyster.

# Stock development through genetic manipulation \_\_\_\_\_

According to Hershberger et al. (1984), two developments of Pacific oyster culture in the United States have made selection and direct breeding both feasible and attractive. First, a successful artificial spawning technique for the Pacific oyster and development of a procedure for larval rearing provide the means to exercise control over the entire life history. Recently, results from more detailed studies of conditioning and spawning procedures have identified several factors that can improve gamete quality (Lannan 980, Lannan et al. 1980, Muranaka and Lannan 1984) and, thus, predictable larval production.

Secondly, Hershberger et al. (1984) indicated the technology of seed production in commercial oyster hatcheries had developed to a point where their seed has become competitive (in terms of reliability and cost) with seed collected from natural production. It has only been within the past five years that hatchery-produced spat of Pacific oysters has been in demand by the oyster growers (Clark and Langmo 1979).

A systematic selection and breeding program has been conducted with the Pacific oyster at the University of Washington for almost 15 years (Beattie et al. 1978, 1980; Perdue et al. 1981; Hershberger et al. 1984). It was initiated during the 1970s when there were major summer mortalities occurring along the Pacific coast which necessitated a look at the possibility of breeding for a strain of oysters resistant to summer mortalities. Histological studies into the mortalities revealed there was no identifiable pathogen that could be clearly related to the oysters dying in specific bays during the summer. Detailed studies revealed mortalities may be related to the gametogenic cycle and the physiological processes and stresses that take place during that time (Perdue et al. 1981). Thus, several approaches were utilized during the past 15 years at the University of Washington oyster genetics program, focusing on three areas: 1 Survival during summer mortality, 2 genetic determination of carbohydrate (glycogen) content in relation to gametogenic cycles, and 3 the effects of inbreeding.

During the past four years a new focus has been added the development of a triploid oyster. This was pursued because of the need by oystermen for a summer oyster that does not go through full gametogenesis. During the summer months a normal diploid oyster will produce eggs or sperm and become milky. Although edible, the product is aesthetically undesirable and in some cases unacceptable as a half-shell oyster. Thus triploidy has been looked upon as a potential for eliminating or reducing the incidence of gonadal maturation in oysters, affectively neutering them for the fresh oyster trade during the summer.

#### Summer mortality

Although summer mortality has abated in recent years, the approach utilized to study the problem and the breeding program that evolved to attain resistant stocks are worth reviewing. A major part of the early work on this problem focused on identification of the agent responsible for mortality (Glude 1975). Studies in Japan concluded that the summer mortalities were largely the result of physiological stress associated with highly eutrophic conditions (Koganezawa 1975). In both Japan and the United States, mortalities were generally associated with areas of high productivity, high nutrient level, and water temperatures exceeding 20°C, coincident with a period of maximum gonad maturation (Perdue et al. 1981). Laboratory research demonstrated that mass mortality approximating the characteristics of the natural situation could be induced by holding oysters in 20°C water and increasing the nutrient levels (Lipovsky and Chew 1972). Although studies by Grischkowsky and Liston (1974) demonstrated that Vibrio sp. may play a significant role in the laboratory mortality tests, they do not appear to be a causative pathogen under field conditions.

It should be noted that none of the early work in Japan on summer mortality included selective breeding as a method to mitigate the severity of this problem. One has to recognize that the initial information needed before conducting a selection and breeding program is to determine whether the organism contains adequate genetic variability on which to base the program; thus, genetic variability tests through electrophoretic analysis were conducted by Buroker et al. (1975). Their study indicated a good selective breeding potential and led to a selection design and genetic analysis (Fig. 5) used in breeding oysters for resistance to mortality during simulated summertime stress (Lipovsky and Chew 1972) to induce a 60-70% mortality. Survivors were spawned and mated by crossing a single male with a single female to produce experimental families. Progress in increasing resistance in the families produced was measured by challenging offspring when they reached adulthood with the same elevated temperature conditions. Results from selected groups were compared with those using an unselected and control population to assess progress.

According to Hershberger et al. (1984), progeny from the initial crosses performed in 1973 and 1975 indicated a good potential for development of oyster strains resistant to thermal stress (Beattie et al. 1978). Out of the seven families originally tested, two consistently survived the thermal stress significantly better than the control groups and none of the selected families showed poorer survival. Thus, it appeared that selection could be used to improve resistance to one factor involved with summer mortality, thermal stress.



Figure 5 Diagram of selection design and genetic analysis used in breeding oysters for resistance to mortality during simulated summer stress (from Beattie et al. 1978 and Hershberger et al. 1984).

Although laboratory tests proved that resistant strains can be developed, it was necessary to field-test the various families in areas of known summer mortalities. It was during this period, the mid-1970s, that the summer mortalities abated. However, we were able to determine that, although increased gonadal development was not directly related to high or low mortality, the timing of the mortality coincided with maximum gonad development (Perdue et al. 1981). Further, from testing the families when mortalities were still occurring, it was discovered that those with higher survival had consistently higher carbohydrate (glycogen) stored energy reserves than oyster families with lower survival. With this type of information, selected families with higher glycogen levels as well as better shell growth were bred in the hopes of developing an oyster with higher marketability.

## **Carbohydrate content**

Carbohydrate (glycogen) is the major stored energy reserve in oysters and during anaerobiosis is the only substrate utilized for metabolic processes (Hochachka and Somero 1973). In addition, it was pointed out by Gabbott (1975) that gamete production occurs at the expense of stored glycogen reserves. This is the primary reason glycogen levels have been shown to be inversely related to gonadal development in Pacific oysters (Matsumoto et al. 1934, Mann 1979, Hershberger et al. 1984). Perdue et al. (1982) and Hershberger et al. (1984) indicated that heightened gonadal development has a major influence on susceptibility to summer stress conditions and that carbohydrate content may be a more precisely measured trait on which to base selection. Further, carbohydrate content is an important component of oyster marketability. Immature or nonspawning (high glycogen content) oysters have a high market desirability compared with



Figure 6 Rotational line-breeding plan which produces eight full-sib and four half-sib families (from Hershberger et al. 1984).

mature oysters during peak gonadal maturation (low glycogen content). Thus, selection and breeding to maintain high carbohydrate content could also improve the marketability of oysters (Hershberger et al. 1984). Over a ten-year period the breeding program has provided lines with significantly elevated carbohydrate levels, and are currently utilized for broodstock for one company's production of gourmet oysters. Preliminary results on the carbohydrate content of a series of selected families grown in different locations suggest there is a major genetic component in the utilization of a glycogen during gonadal development.

## Inbreeding

Hershberger et al. (1984) discussed the effects of inbreeding in the University of Washington selection and breeding program for oysters. They chose to avoid inbreeding depression by the use of a scheme of rotational line crossing, a systematic breeding design that minimizes the increase in level of inbreeding per generation (Fig. 6). In this rotational design (produces eight full-sib and four half-sib families) the increase in inbreeding coefficient is less than 0.01 per generation. Although this approach does not decrease the amount of inbreeding initially imposed by a small breeding population size, it does minimize the change across generations. It should be noted that recent preliminary results from some of the family bred for high glycogen levels (Beattie et al. 1986) suggest inbreeding depression by exhibiting poorer shell growth. This is presently being addressed in our breeding studies, and the success from using this breeding design is still being assessed.

## Triploidy

Recent work by two researchers, Standish K. Allen and Sandra L. Downing at the School of Fisheries of the University of Washington, has shown conclusively that triploid Pacific oysters can be produced as a viable commercial option (Allen 1986, Allen and Downing 1986, Downing and Allen 1987). Allen (1986) indicates that triploidy has been produced in shellfish using three different methods: Chemical, pressure, and thermal induction. All three affect inhibition of polar body development resulting in an additional maternal set of chromosomes. Allen pointed out that all methods to induce triploidy depend upon absolute control of the moment of fertilization and subsequent meiotic events in the egg. Since the rate of these events is temperature-dependent, reproducibility of induction procedures in a given species will depend on maintaining constant temperature during incubation. Studies by Downing and Allen (1987) clearly demonstrate the dependence of temperature on time in which treatment can take place. In their studies they use primarily Cytochalasin B (CB) to induce triploidy. Although earlier work with hydrostatic pressure has been shown to produce triploidy in Pacific oysters (Chaiton and Allen 1985), the results were not as good as with CB.

Researchers Allen and Downing report that treatment consists of adding 1 mg of CB dissolved in 1 mL of dimethylsulfoxide (DMSO) to a liter of sea water containing fertilized eggs at the beginning of any treatment. After 15 minues, the eggs are filtered through a 25- $\mu$ m screen and then resuspending the zygotes in a 0.1% DMSO bath for another 15 minutes. During this time, egg suspensions are stirred occasionally during the treatment and rinsed. After rinsing, zygotes are placed in appropriate tanks and reared according to accepted procedures (Breese and Malouf 1975). Studies by Downing and Allen (1987) demonstrate conclusively that the time and temperature of treatment application are very critical (Fig. 7). Each of the Roman numerals in this figure represents 15-minute intervals after fertilization of the eggs and the time at which each individual test on different lots were made. The fitted curves reveal that the triploid induction maxima at 18°, 20°, and 25°C were 52, 76, and 90%, respectively. Also, lowering the temperature delays the induction peaks; maxima at 25°, 20°, and 18°C are approximately 30, 45, and 50 minutes postfertilization, respectively. Two private hatcheries are now utilizing these techniques to produce triploidy Pacific oysters, and both have them already available for the half-shell oyster market, especially for the summer periods. Further, there are now available a hatchery manual and accompanying video by Allen et al. (1989) for producing triploidy oysters.



#### Figure 7

Induction profiles at 18°C (top), 20°C (middle), and 25°C (bottom) were produced by fitting curves to data using multiple linear regression. Derived equations and correlation coefficients are shown. Points represent percent triploidy in each treatment group. Treatment periods are 15-min intervals beginning at fertilization. (Downing and Allen 1987)

## **Concluding statement**

An attempt has been made to briefly summarize the recent changes in oyster cultivation along the west coast of North America. Although intertidal bed cultivation is still the most important, attempts have been made to implement a variety of off-bottom culture techniques in many select areas. Offbottom culture is growing, but continues to encounter difficulty because of permit application requirements related to the sociopolitical climate existing in most populated areas in the United States. This is especially true for applications for raft or floating type culture facilities.

There is no doubt that the new concept of remote setting of eyed larvae for Pacific oysters will grow. Less than 10 years ago the oyster farmer on the west coast still needed to be concerned with getting adequate supplies of seed oysters. Now the natural catches in several areas, complemented by many farmers building setting tanks on their own property to catch their own seed through remote setting, have removed this concern. In essence, some growers no longer depend on natural catches because of remote setting for their own seed. The cost is equivalent according to some growers.

Two hatcheries, Coast Oyster Hatchery in Washington and Whiskey Creek Hatchery in Oregon, were expected to produce over 12 billion eyed larvae for sales or for their own use in 1986 to satisfy the needs of the west coast industry. There are other hatcheries in California and British Columbia, and one being proposed for Alaska.

Stock development through genetics and breeding studies have been in existence on the west coast for more than 10 years. The University of Washington School of Fisheries has been investigating summer mortalities of Pacific oysters in the late 1960s and early 1970s and attempting to breed a resistant stock to this disease. No known pathogen was found consistently related to this summer kill. Further, studies strongly suggested that the phenomenon was related to physiological stress related to the reproductive cycle. Stocks of oysters that died generally had lower carbohydrate (glycogen) levels. With this in mind, a breeding program was established to develop stocks with higher glycogen levels and including an approach to minimize inbreeding problems. Although attempts were made to reduce this problem, early results show continued breeding of summer oysters for high glycogen can also lead to oysters with slow shell growth. This problem is presently being addressed at our experimental hatchery.

The production of triploid Pacific oysters is looked upon very favorably by the shellfish growers on the west coast of North America. Researchers at the University of Washington School of Fisheries have been instrumental in the development of the triploid oyster. The fact that these triploid oysters have minimal gametogenesis as compared with normal diploid oysters and, thus, more carbohydrates in the tissues during summer, ensures that these neutered oysters will be in great demand for the half-shell oyster trade during the summer months.

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## A Physiological Approach to Problems of Mass Culture of the Rotifer

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## ABSTRACT

The introduction of the rotifer *Brachionus plicatilis* as a food organism in 1960 has made such remarkable progress in the mass-culture techniques of marine fish fry that, for instance, about 40 million red seabream young were produced in Japan in 1984. Strains of rotifer used in Japanese hatcheries are roughly divided into two groups: L type and S type, according to differences in morphology and in growth response to environmental temperature. The rotifer has high food selectivity. Whereas living materials are usually acceptable, nonliving materials are rejected by the rotifer, although there are many exceptions. Greater attention to food selectivity is needed when attempting to develop new kinds of food.

The dietary value of several kinds of phytoplankton were compared with marine Chlorella by conducting parallel tests under bacteria-free conditions. Green or blue-green algae were found to be excellent food, while diatoms had a low dietary value. The nutritive value of baker's yeast was found to be extremely low when tested under bacteria-free conditions with pure washed yeast cells. Therefore, the success of mass culture of the rotifer fed only yeast is due to byproducts of decomposition or to the growth of phytoplankton or bacteria in culture tanks which become a supplementary source of the nutrient lacking in yeast, such as vitamin  $B_{12}$ .

The rotifer requires fat-soluble vitamins A, D, and E as essential nutrients. The rotifer can tolerate relatively low oxygen level. Low oxygen concentration is apparently suitable to rotifers fed with yeast because the bacteria which produces vitamin  $B_{12}$  in culture tanks usually belongs to facultative anaerobic bacteria.

Un-ionized ammonia accumulating in culture tanks is probably one of the causes of suppressed growth of the rotifer. Also pH may have an indirect rather than a direct effect on rotifer growth through un-ionized ammonia. Since the introduction of the rotifer *Brachionus plicatilis* to the seedling culture as a food organism about 25 years ago (Ito 1960), mass-culture techniques for marine fish fry have progressed so remarkably that, for instance, two kinds of fish can be produced in numbers exceeding 10 million per year (1984) as seedlings for mariculture and for releasing to the coastal waters (red sea bream, 39.3 million; flat fish, 13.7 million). Feeding schedules of seedlings were classified into four types according to size of larvae or type of required foods (Fig. 1) (Kitajima 1985). The only fish seedlings that can be produced on a mass-production scale are the standard type or the bottomfish type which can utilize the rotifer as the major food throughout their larval stage. This fact emphasizes the importance of the rotifer as a food organism.

Table 1 shows examples of red sea bream, Pagrus major, production in typical hatcheries in Japan. An average hatchery can produce more than 1 million young (>10 mm total length) per year. However, as shown in Table 1, compared with the tank volume needed for culturing fry, a much larger tank volume is needed for rotifer production and an even larger volume for marine Chlorella culture. There have been many attempts to develop artificial food for fry, and some of them are already being produced on a commercial scale. However, these cannot completely replace the rotifer but only function in a supplementary role. Therefore, the rotifer is and will remain the main food for fry production during the next decade. Government officials project that the demand for young red sea bream for release and mariculture will reach 50 million or more in 1987 (Aihara 1984). Twice the present rotifer production will be required for the release of artificially produced fish fry into coastal waters to effectively increase coastal resources and to supply enough fry for mariculture.



Figure 1 Four types of feeding schedules for larval and juvenile stages of many useful marine fishes.

		Seedling p	Table 1           Seedling production of red sea bream in typical sea-farming centers in Japan.											
		A		В	С									
		Num		Tank volume	Number rotifers sur		Tank volume used for rotifers	Tank volume used for Chlorella						
Hatchery	Year	Year $(\times 10^3)$ (A/I	(A/B)	(m <sup>3</sup> )	(× 10 <sup>4</sup> )	(C/A)	(m <sup>3</sup> )	(m <sup>3</sup> )						
I	1981	1043.7	(5.8)	180	4,111,000	(3.9)	1200	2400						
I	1982	3126.0	(9.9)	315	25,377,000	(8.1)	1200	2400						
I	1983	3033.0	(11.2)	270	18,337,000	(6.0)	1200	2400						
п	1983	3240.0	(6.2)	520	30,832,000	(9.5)	672	2170						
ш	1983	2780.0	(2.8)	1000	19,670,000	(7.0)	313	1430						

C		able 2 two strains of rotifer	•	
		Lorica	Water ten	nperature (°C)
Туре	Length µm	Shape (anterior spine)	Suitable	Lower limit for growth
S (B. plicatilis rotundiformis)	150-220	Round (pointed)	28-35	20
L (B. plicatilis typicus)	200-360	Slender (obtuse angled)	18-25	10

This paper presents a physiological approach to present problems in improving mass culture techniques of the rotifer.

## **Rotifer variations**.

After introduction of the rotifer into mass production of fry, early studies on the physiological responses of rotifers to environmental conditions ignored variations between strains. However, recently the existence of great variations among rotifers has become recognized. In Japan, strains of rotifers used in hatcheries were roughly divided into two groups-L type and S type-according to size and shape of the lorica and shape of the spines of the anterior lorica (Fukusho 1983). The two strains also have different growth responses to environmental temperature. Table 2 summarized the morphological and physiological differences between L and S types. S-type rotifers are suitable for rearing of smallmouthed larvae such as groupers and rabbit fishes. On the other hand, L-type rotifers are best for large-mouthed larvae. Recent studies have shown that the S and L types should be divided into different genetic strains and classified taxonomically as subspecies B. plicatilis typicus and B. plicatilis rotundiformis, respectively (Suzuki 1983). Studies outside Japan reported the varieties in B. plicatilis that show genetic as well as morphological variation in the electrophoretic pattern (Serra and Miracle 1983, 1985; Snell and Carrillo 1984; Snell and Winkler 1984). Therefore, we should study genetic differences between the two types by electrophoretic procedures. These studies may add fundamental knowledge to efforts to develop very small or very large strains by selection or hybridization, strains suitable for rearing small- or large-mouthed fry.

## Search for suitable food \_

The food material usually used in rotifer mass culture is marine Chlorella. However, recent investigations have revealed that some phytoplankton used as Chlorella in Japanese hatcheries belong to the family Eustigmatophyceae, genus *Nannochloropsis* (Maruyama et al. 1986). As mentioned above, production of Chlorella requires a huge tank volume and sometimes the supply is unstable. Therefore, efforts have been undertaken to find suitable food material to replace Chlorella. One approach is to culture the rotifer with the objective food on a mass-culture scale. *Tetraselmis tetrathele* has been introduced as a good suitable food algae (Okauchi and Fukusho 1984). Baker's yeast and marine yeast have been practically utilized (Hirata and Mori 1967,

	Filter	Table r feeding of the rotife	e 3 r Brachionus plicatilis.	
Food	Density (cells/mL)	Water temperature (°C)	Filtration rate (µl/h/ind.)	Reference
Dunaliella salina	$(5.0-14.4) \times 10^4$	20	0.64-1.5	Doohan 1973
Synechococcus sp.	$8 \times 10^{6}$	26.1-28.1	3.0	Ito in Doohan 1973
Chlorella sp.	$213 \times 10^{4}$	25	4-6	Hirayama and Ogawa 1972
Chlamydomonas sp.	$10 \times 10^{4}$	23	8.4	Chotiyaputta and Hirayama 1978
Olisthodiscus sp.	$5 \times 10^{4}$	23	2.0	Chotiyaputta and Hirayama 1978
Protozoa	$1.75 \times 10^{5}$	25	8.1	Hino et al. 1981a
Bacteria	$1.91 \times 10^{7}$	25	3.4	Hino et al. 1981a
Dunaliella tertiolecta	$1.03 \times 10^{4}$	?	10	Deway in Starkweather and Gilbert 1977
Activated sludge	1	25	0.33-5.45	Hino and Hirano 1980

	Frequ	iency (movemei	Tal nt/min) of mast	ble 4 ax movement of	f rotifers after	feeding.		
	Chlamydomonas sp.			Fo	od		Seawater	
Food	Avg.	SD	t test	Avg.	SD	t test	Avg.	SD
Baker's yeast	68.0	23.6		62.9	23.4	***	22.1	11.6
Pavlova lutheri	65.5	34.3		74.0	31.2	***	33.3	20.0
Cyclotella cryptica	44.6	27.8		53.2	27.7	***	11.4	10.5
Gluten	66.1	22.4		58.1	15.7	***	26.0	16.3
Egg albumin	70.1	30.2	*	54.5	15.7	***	31.5	17.5
Milk	66.1	22.4	*	51.2	21.3	***	29.0	16.3
Corn starch	78.1	21.2	**	60.4	15.8	***	24.4	17.1
Olisthodiscus sp.	58.8	26.5	***	25.7	15.7		20.6	11.2
Linoleic acid	79.6	25.4	***	32.0	12.1		34.4	16.7
Oleic acid	79.6	25.4	***	20.0	16.6	**	34.4	16.7
Culture medium	70.1	30.2	***	39.5	13.4		31.5	17.5

Furukawa and Hidaka 1973). Bacterial flocs (Yasuda and Taga 1980), photosynthetic bacteria (Sakamoto and Hirayama 1983), activated sludge (Hino et al. 1981a, b), dry Chlorella (Hirayama and Nakamura 1976), concentrated Chlorella, and AMT flocs (Fukuhara et al. 1982, Higashihara et al. 1983) have been examined. AMT is the residue of distillation of alcohol produced by fermentation of molasses. AMT floc means microbial flocs produced from AMT plus potassium phosphate with strong aeration. Formulated food for the rotifer is now under development (Gatesoupe and Luquet 1981; Gatesoupe and Robin 1981, 1982).

Another approach is to investigate the physiological response of the rotifer to food, such as food selectivity and nutritional requirements. Table 3 presents data on the filtering rates of rotifers obtained by using suspensions of many kinds of particles (Hirayama 1983). The broad distribution of data suggests that the rotifer is highly selective of food, though strains employed in the experiments may differ. Food particles that reach the mastax have passed three barriers for food selection, as explained with B. cariciflores (Gilbert and Starkweather 1977). Therefore, after feeding various kinds of food particles, the frequency of mastax movement may reflect food selectivity. Table 4 presents frequencies of mastax movement after feeding many kinds of food particles, together with frequency data of the same individuals fed the preferred Chlamydomonas sp. and, as controls, only filtered seawater containing no food particles (Funamoto and Hirayama 1982). Differences between frequencies for each food and for controls appear to be due to food selectivity. Many living materials are acceptable to the rotifer, while nonliving materials are rejected, although there are many exceptions. We must pay more attention to food selectivity as we attempt to develop new kinds of food, because rejected food causes severe problems by fouling the culture water, even if the nutritive value is complete for rotifer growth.

## **Culture methods**

In order to evaluate the nutritive effects of foods or supplementary effects of nutrients added to the basic food suspension, batch culture and individual culture methods were employed. When necessary, rotifers were cultured under bacteria-free conditions. The simplest is the batch culture method in which offspring of the first-laid eggs hatched in one day are cultured in test tubes, each containing four or five individuals with the specific food to be tested. During cultivation, the culture water is not replaced and no additional food is added to the tubes. After several days of culture, the increase in the number of rotifers is determined and the effect on population growth evaluated by comparing the change in numbers of individuals.

In individual culture, first-laid eggs are cultured separately in many test tubes, each containing two individuals under defined conditions. These are observed daily with renewal of food suspension, and the numbers of surviving individuals and of eggs laid are counted. From daily survival rate and fecundity data, two indices-net reproduction rate and intrinsic rate of population growth-are estimated by Birch's method (Birch 1948). The values of indices calculated represent the growth phase of a group according to the fecundity schedule obtained by the individual culture method. Net reproduction rate (Ro) is the number of eggs laid by an average female in her lifetime or the rate of multiplication in one generation. Intrinsic rate of population increase (r)is the constant in the differential equation of population increase, dN/dt = rN, in an unlimited environment (N = number of animals, t = days elapsed since beginning of testtube culture).

The advantage of evaluation by batch culture is that the culture conducted through several generations makes the influence of physiological condition during stock culture negligible. However, the batch culture has its disadvantage in terms of the difficulty in keeping the density of food suspension constant.

#### Nutritional comparisons

Several kinds of phytoplankton were compared with marine Chlorella for nutritional value by the parallel test of individual cultures under bacteria-free conditions, using the first-laid eggs derived from an actively growing group (Hirayama et al. 1979). The nutritional value of each plankton was evaluated according to the ratios of *r* and *Ro* and compared with those obtained with Chlorella. The relative values of many phytoplankton shown in Figure 2 indicate that green or blue-green algae are usually excellent food. *Tetraselmis tetrathele*, which was recently recommended for use as a food for rotifer mass culture, possesses a higher nutritive value for rotifer growth (Hirano and Hirayama 1984). The fact that diatoms have a low nutritive value agrees with the observation that the propagation of diatoms in mass-culture tanks sometimes suppresses growth of the rotifers.



**Figure 2** 

Relative values (closed symbols) of two indices for each phytoplankton compared with dietary effect of marine Chlorella. Open symbols show relative values for *Cycrotella cryptica* calculated against those of *Dunaliella tertiolecta* in a parallel experiment using the two species.



Figure 3 Effect of supplementary vitamin B<sub>12</sub> on rotifer numbers in suspension of baker's yeast in batch culture, 23°C.

The nutritive value of baker's yeast was examined under bacteria-free conditions. Tests by both methods for nutritive evaluation indicate a nutritive deficiency of the yeast (Fig. 3, Table 5) (Hirayama and Funamoto 1983). The eggs laid are

not viable, and the rotifers exhibited no growth. Addition of vitamin B<sub>12</sub>, however, can improve the nutritive value of baker's yeast so that, along with the supplement of vitamin  $B_{12}$ , it can support the growth of the rotifer. However, values of indices by two evaluation methods are still much lower than those in marine Chlorella suspension. In spite of extremely low nutritive value of baker's yeast, there are many cases in which the successful mass culture of the rotifer was achieved by feeding only baker's yeast as a food source. The nutritive test on the decomposed marine yeast, and on the addition of marine Chlorella at extremely low density to a marine yeast suspension, revealed that the success of mass cultures of rotifers fed with baker's yeast alone may be improved by the byproducts of decomposition or by the growth of phytoplankton or bacteria in the culture tanks (Hirayama and Watanabe 1973). These provide a supplementary source of the nutrients lacking in yeast, such as vitamin  $B_{12}$ . These supplementary effects are also supported by the fact that,

of baker's yeast	Table 5           ned by individual culture of n           (50 µg/mL) supplemented           arious concentrations at 23	with vitamin B <sub>12</sub> in
Concentration	Intrinsic rate of	Net reproduction
of vitamin B <sub>12</sub> (µg/mL)	population increase (r)	rate (Ro)
0	0.002	0.70
0.01	0.03	1.20
0.05	0.25	3.78



#### **Figure 4**

Contents of vitamin  $B_{12}$  in culture water, (A) filtrates of AMT and AMT floc, (B) in several kinds of food for the rotifer, and (C) in the bodies of rotifers produced by feeding that food (from Imada 1984).

although several kinds of food such as baker's yeast strengthened with essential fatty acid ( $\omega$  yeast) contain little vitamin B<sub>12</sub>, the bodies of the rotifers grown successfully on such diets contained high levels of vitamin B<sub>12</sub> (Fig. 4) Imada 1984).

In Kaiike, Kagoshima prefecture, photosynthetic bacteria growth supports feral rotifers as a main energy source (Matsuyama and Shirouzu 1978). After isolation of the bacteria, the nutritive value of such bacteria to rotifer growth was examined. The photosynthetic bacteria could not support high growth of the rotifer, while an extremely low-level supplement of marine Chlorella was effective in strengthening the nutritive value of this bacteria (Sakamoto and Hirayama 1983).

As mentioned above, improvement of the nutritive deficiency of baker's yeast by supplement of vitamin  $B_{12}$ indicates that vitamin  $B_{12}$  is one of the essential nutritive elements to the rotifer as shown by Scott (1981). Nutritive requirements of the rotifer for fat-soluble vitamins were examined by means of supplying each of them to the basic food suspension of baker's yeast plus vitamin  $B_{12}$ . The results by individual and batch cultures are shown in Figure 5. Each addition of vitamins A, D, and E was effective on rotifer growth, and combinations of these vitamins are more effective than addition of a single vitamin. These results indicate that the three vitamins are essential nutritive elements for growth of the rotifer (Satuito and Hirayama 1986).

## Environmental conditions regulating rotifer growth

As mentioned above, the response of rotifer growth to temperature is different in S and L strains (Table 2). L-type rotifers tolerate relatively lower temperatures; in contrast, S types are adjusted to relatively high temperatures (Ito et al. 1981). Although other physiological responses to environmental conditions may be reflected by strain differences, there has been no study in which close attention was paid to strain differences. On osmoregulation in the rotifer, it has been shown that this species has a high ability to tolerate a wide range of external concentrations of salts, and that the lack of ability to regulate hyposmotically at concentrations approaching full-strength seawater suggests a marine ancestry for this animal (Epp and Winston 1977). Observations in mass-culture tanks showed that appropriate pH values for mass culture of the rotifer ranged from 7.1 to 7.5 (marine yeast) and 7.5 to 8.1 (baker's yeast) (Furukawa and Hidaka 1973). However, Epp and Winston (1978) revealed by their laboratory culture that a wide range of pH, from 6.5 to 8.5, had no direct harmful effect on the rotifer population growth. Yu and Hirayama (1986) pointed out that un-ionized ammonia accumulating in the culture water could be one of the causes of sudden decrease or suppressed growth of the rotifer, and that high pH value may not directly, but may indirectly, influence rotifer growth through un-ionized ammonia concentration.



#### Figure 5

The supplementary effect of fat-soluble vitamins on the growth of the rotifer. Vitamins were added to the basic food suspension consisting of 200  $\mu$ g/mL of baker's yeast and 1.4  $\mu$ g/mL of vitamin B<sub>12</sub>. (A) Relative *r* and *R<sub>o</sub>* for each fat-soluble vitamin at different concentrations in individual culture; (B) Total increase in population of the rotifer in food suspensions supplemented with fat-soluble vitamins at different combinations in batch culture.

The rotifer can tolerate relatively low oxygen levels. Imada (1984) observed an inverse relationship between the oxygen content of mass-culture tanks and the growth rate of rotifers fed with AMT flocs or  $\omega$  yeast (baker's yeast strengthened with essential fatty acid) (Fig. 6). This appears to indicate that the rotifer grows better at low oxygen levels. However, this was not the case when marine Chlorella was provided as a food. It seems likely that this relationship occurred because the bacteria which produced vitamin B<sub>12</sub> in yeast-fed cultures are facultative anaerobic bacteria.

In mass culture of the rotifer there are many cases of sudden decrease or suppressed growth of the population. Some of these could be explained by physiological responses of the rotifer as described above. However, we have many cases which seem to have no relation to the causes mentioned



#### Figure 6

Relations between oxygen content and growth rate of two groups of rotifer, one fed only AMT floc and one only  $\omega$ -yeast (from Imada 1984).

above. An abrupt change of water temperature may directly or indirectly affect the growth of a rotifer culture. Suppressed growth is often observed during a period of shift of dominant strains. Also, the fomation of large numbers of dormant eggs sometimes results in a sudden decrease of the rotifer population. We often observe that suppressed growth is accompanied by an increase in diatoms or protozoa in the cultures.

## Problems for future study \_

Many problems remain to be solved to establish more reliable rotifer culture techniques: 1) Select or produce the suitable size of rotifer with a faster and more stable growth, 2) establish reliable mass-culture techniques of marine Chlorella or find substitute phytoplankton, 3) clarify the nutritonal requirements and to formulate completely artificial diets for the rotifer, 4) completely clarify the environmental conditions regulating population growth, and 5) understand the factors controlling bisexual reproduction.

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Effects of Environmental Instability on the Growth of the Japanese Scallop *Patinopecten yessoensis* in Abashiri Sowing-Culture Grounds

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## ABSTRACT

Environmental instability in Abashiri waters is caused by many factors, including geographical features, hydrographic structure, drift ice, and atmospheric conditions. Therefore, the degree of instability varies year to year. The growth of scallops is affected by environmental instability, especially by temperature fluctuations which depress feeding activities spring to summer. Then, growth is slower than in the western part of the Okhotsk Sea coast, and fluctuates according to the degree of environmental instability each year. It is difficult to minimize such environmental effects on the growth of scallops. However, the recatch:release ratios of sown scallops have increased year after year to 40%. This demonstrates that reasonable methods can bring about an abundant harvest even in inadequate environments such as Abashiri Bay. Along the Okhotsk Sea coast of Hokkaido in Japan (Fig. 1) before 1973, production of the Japanese scallop *Patinopecten yessoensis* depended on natural resources. Until 1945, the annual yield of scallop fluctuated markedly, but the highest yield reached 15 thousand tons in Sarufutsu and other grounds. However, yields decreased each year after 1945. In 1973, sowing-culture began on a large scale in Sarufutsu. This sowing-culture was a great success, reaching an annual yield of 40 thousand tons in 1984. Subsequently, sowing-culture has spread along the Okhotsk Sea coast.

In Abashiri Bay (Fig. 1) sowing-culture began in 1978. In 1980, however, high mortalities and markedly slow growth of sown scallops were observed. We have studied the growth and survival of scallops in Abashiri sowingculture grounds from 1982 to 1984.

## Sowing-culture on the Okhotsk Sea coast \_\_\_\_\_

The Sea of Okhotsk is a semi-closed sea surrounded by land and islands and has four distinct characteristics. (1) The surface layer above 50 m depth, the Okhotsk Surface Water, has very low salinity ( $<32.5^{\circ}/\infty$ ). (2) Drift ice develops and covers 80% of the Sea of Okhotsk in winter. (3) The Soya Warm Water, of high temperature and high salinity, enters into this sea through the Soya Straits (Fig. 1) from the Sea of Japan. The Soya Warm Water flows along the coast of Hokkaido and reaches the Siretoko Peninsula March to October. (4) The continental shelves are as wide as 200 km in the western part of this coast and become narrower to the east, being only 16 km off Abashiri Bay.

Squares marked A, B, C, and D (Fig. 1) are rotating sowing-culture grounds. Measurements of water quality and other elements were carried out at Station A in Abashiri Bay and Station D off Lake Notoro (Fig. 1).

In these sowing-culture grounds, the removal of starfish is carried out by dredging. After this, from 60 to 100 million scallop juveniles, 1 year old, are sowed in June. After 2 years, the sown scallops are harvested.

In Abashiri A, sowing-culture began on an industrial scale in 1978. However, it was found in 1980 that the survival ratio was only 4.2% and growth was slower than on the other grounds of the Okhotsk Sea coast. Total wet weight of sown scallop at 3 years of age in Mutsu and Funka Bay, Okhotsk Sea coast, and Abashiri A were 250, 170, and 80 g, respectively. Thus scallops in Abashiri Bay grew to only one-third the weight of those in Mutsu and Funka Bay.

Scallops have been known to grow well in Abashiri D, as in other grounds located in the western part of this coast; however, they grow poorly in Abashiri Bay. Therefore, we



Figure 1

Location of the scallop grounds in the study area. Squares marked A, B, C, and D are rotating sowing-culture grounds.



Figure 2 Schema of hydrographic structure generally observed immediately after the disappearance of drift ice from the Okhotsk Sea coast.

expected to find distinct differences in environmental conditions between Stations A and D. However, the results of many water-quality measurements did not differ between Stations A and D during 3 years. Therefore, we need to consider other factors contributing to the inadequate environment for scallops in Abashiri Bay.

## **Environmental factors**

## Winter drift ice

Drift ice causes extremely low water temperature, which represses the growth of scallops. The duration of the drift ice period varies markedly between years, from 2 to 5 months in Abshiri, depending on climatic conditions.

## Spring upwelling

After the disappearance of drift ice from this coast in March or April, the Okhotsk Surface Water spreads over the surface layer (Fig. 2). Along the western part of this coast, from Soya Straits to near Lake Saroma, the Soya Warm Water (S.W.W.) occupies the entire water column and flows alongshore. Approaching Abashiri Bay, the S.W.W. sinks along the deepening bottom, and then, off Abashiri Bay, the S.W.W. flows along the bottom at a depth of 100 or 200 meters. These hydrographic structures are caused by the higher density of the S.W.W. and the deeper bottom around Abashiri Bay. In any case, the S.W.W. rises to the surface in summer. We can assume two processes which raise the S.W.W. to the surface.

One of these processes is the decreased density in accordance with the rise in temperature of the S.W.W. With this process only, it takes 2 or 3 months for the S.W.W. to reach the surface layer. Therefore, temperatures in the coastal water rise gradually, as shown in Figure 3, Station A, in 1982.

Another process is wind-induced upwelling. In this region, upwelling induced by the east wind moves the surface water offshore. Although the east wind is very small in this region, the south wind also moves the surface water offshore.

In the spring of 1983, marked upwelling occurred with the following processes. A strong south wind blew continuously for more than a week from the end of March to early April and moved the drift ice offshore from Abashiri Bay. At the same time, the surface water of the coast was transported offshore. Then the bottom water, the S.W.W., was transported to the shoreline. Thus, the temperature rose rapidly along the coast (Fig. 3). At the same time, the spring phytoplankton bloom was removed and replaced by water containing low levels of nutrients (Fig. 4). Along this coast,



Figure 3 Seasonal temperature variations at 30-m depth at Abashiri Stations A (upper) and D (lower), 1982–84.







Figure 5 Daily mean temperature variations (dotted line) at 28-m depth of Abashiri Station D measured with Ryan Model J, and daily mean wind speed (solid line) observed by Abashiri Regional Meteorological Observatory, April-September 1984.

a phytoplankton bloom is expected to develop once a year, although the scale of the spring bloom fluctuates widely. The largest amoung of chlorophyll *a* was 270 mg/m<sup>2</sup> in 1984, and the smallest was 60 mg/m<sup>2</sup> in 1983 (Fig. 4).

The scallop spawning season is May to June in this area. Spring phytoplankton bloom is thought to be the major food source for scallops just prior to spawning. These fluctuations of spring bloom are considered to be a disadvantage for the spawning of the scallops.

The same phenomena were observed in the next year, 1984. From April to June, three temperature peaks were observed (Fig. 5), caused by upwelling of the S.W.W. induced by strong south winds. Thus, the S.W.W. was brought up to the upper layer. At the period indicated by the arrow in Figure 5, the S.W.W. reached the surface. After this period, the upper layer of the water mass was replaced by the lower, and this upwelling caused the sudden fall in temperature.

## Summer temperature fluctuation

After reaching the surface, the S.W.W. flows alongshore, occupying the entire water column off Lake Notoro, and temperature gradients extend vertically (Fig. 6). The same structures are observed generally in the western part of this coast. It is believed that this hydrographic structure prevents upwelling and temperature fluctuations. On the other hand, off Abashiri Bay, the S.W.W. occupies the upper layer from the surface to about a 100-meters depth (Fig. 6). The temperature gradients extend horizontally. The area between the bottom of the S.W.W. and the sea bottom is occupied by cold water. Under this structure, upwelling causes a sudden temperature drop and many other mechanisms cause temperature fluctuations on this coast.

In August of 1984, rapid temperature changes were observed most frequently (Fig. 7). Daily cyclic changes ranging from 3 to  $5^{\circ}$ C were observed in early August, probably caused by internal tides under the two-layered structure shown in Figure 6. The rapid fall in temperature of  $13^{\circ}$ C during 20–25 August was caused by upwelling induced by a strong south wind. These rapid temperature changes are the main factors that cause environmental instability.

It is clear that daily temperature ranges are wide from May to mid-September. Then, after mid-September, the daily range becomes narrow (Fig. 5) because the layering structure of the water masses is destroyed by the cooling of the surface water.

### Intermediate cold water in summer

Finally, environmental instability along this coast is due to the approach of the Intermediate Cold Water (I.C.W.) to the coast. This I.C.W. is of very low temperature, from -1.8 to  $+2^{\circ}$ C, and exists below the 50-m depth even in summer.



Figure 6

Schema of hydrographic structure generally observed in summer along the Okhotsk Sea coast. S.W.W. = Soya Warm Water; I.C.W. = Intermediate Cold Water; bold lines = temperature gradients.



Figure 7 Two-hour temperature variations at 28-m depth of Abashiri Station A measured with Ryan Model J, August 1984.

Figure 8 shows the hydrographic structure during September of 1984. Off Lake Notoro, the front of the I.C.W. is usually located 25–40 miles offshore (Ohtsuki 1983). However, it was only 9 miles offshore in 1984, the closest approach to shore in 18 years. Then, the S.W.W. condition became unusual, that is, the temperature gradients extended horizontally off Lake Notoro as off Abashiri Bay. This hydrographic structure easily caused the temperature fluctuation nearshore. On this coast, temperatures generally do not fluctuate; however, it seems that temperature fluctuations should occur once every 10 or 20 years by the approach of I.C.W. to this shore.





# In situ biodeposition rates and growth \_\_\_\_\_

In order to understand the relationships between environmental conditions and feeding activities, *in situ* biodeposition rates of scallops were measured. The term "biodeposits" coined by Haven and Morales-Alamo (1966) means "feces and pseudofeces" which are produced by filter-feeding animals, such as scallops, and deposited on the bottom.

In situ biodeposits were collected with sampling apparatus designed by us (Fig. 9). Two-year-old scallops were freshly caught from the bottom of each station. One apparatus with four scallops and one control apparatus without scallops were placed on the bottom. After one day, biodeposits were collected and dry weight measured.

It was expected that biodeposition rates would increase with increases in the amount of food. However, the results did not show such relations (Fig. 10). It is concluded that the extremely low biodeposition rates obtained in ice-covered Lake Notoro in winter (open circles, Fig. 10) resulted from depressed feeding activities caused by the extremely low temperature  $(-1.6^{\circ}C)$ . We consider that biodeposition rates below 200 mg/individual per day are also depressed by environmental factors perhaps related to the rapid and frequent changes in temperature in Abashiri waters.

In consideration of some studies on thermal adaptation (Somoero 1969, Dickie and Medcof 1963), we have assumed that aquatic poikilotherms, such as scallops, that live on the

> Figure 9 Sampling apparatus used for collection of biodeposits of Japanese scallops.







Figure 11

Figure 10 Biodeposition rates of 2-year-old scallops in relation to chlorophyll *a* of ambient water in Abashiri Bay (●) and ice-covered Lake Notoro (○), 1982–84.

Biodeposition rates of 2-year-old scallops (vertical columns) at Abashiri Stations A (left) and D (right). Scallops used were captured at Station A. Temperatures were measured with Ryan Model J (solid line) and with water sampled (dashed line). Horizontal lines show sampling periods of biodeposits. Amounts of chlorophyll *a* were obtained at the bottom of Stations A and D on 23 and 27 July 1984.

sea bottom cannot adapt quickly to rapid changes of temperature. We examined this assumption by *in situ* measurements. The biodeposition rates obtained at Station A with scallops caught freshly at the same place were 180 mg/day on 23–24 July, and decreased to 138 mg/day with an increase in temperature of 3°C during three successive days (left, Fig. 11).

The right graph in Figure 11 shows the measurements at Station D with scallops caught at Station A. During the period 23-24 July, scallops were exposed to a sudden rise and fall in temperature over the range of about 5°C and the biodeposition rate was reduced to 40 mg/day. This is only 20% of that of Station A and as low as that caused by the extremely low temperatures. During three successive days, the biodeposition rate increased a little, but under these fluctuating temperature conditions, it was only half that of Station A.

From the results above and knowledge of the effects of thermal changes, we believe that feeding activities are frequently depressed by fluctuating temperatures from spring to summer in Abashiri Bay.

#### Seasonal variation of biodeposition rates

Seasonal variations in amount of biodeposits are calculated with biodeposition rates obtained in Abashiri waters (Fig. 12). The numbers in every column in Figure 12 indicate the amount of biodeposits produced during every period. From September to December, temperature fluctuations are not so marked, as mentioned previously, and the scallops feed constantly in the stable environment. However, even these amounts of biodeposits are not large, because of the small amounts of food available during this season. In Mutsu Bay where the scallops grow faster than in Abashiri



Figure 12 Seasonal variations in amounts of biodeposits of a 2-year-old scallop in Abashiri Bay, 1982–84.

Bay, the biodeposition rates were almost identical with that of Abashiri Bay in this season (Fuji and Hashizume 1974). In winter (January-April), the extremely low temperature over a fairly long period depresses feeding activities in Abashiri waters. In Mutsu Bay, the most active feeding is done in this season, and the amount of biodeposits reach 80.9 g (Fuji and Hashizume 1974). This active feeding in Mutsu Bay is supported by the spring phytoplankton bloom and by higher temperatures (4-8°C) than Abashiri waters. From spring to summer in Abashiri waters, the amount of biodeposits increases slightly, but it is still small, caused by temperature fluctuations as mentioned previously. Thus, the annual amounts of biodeposits produced by a 2-year-old scallop in Abashiri and Mutsu Bay are 59.4 and 151 g (Fuji and Hashizume 1974), respectively. The Abashiri: Mutsu ratio is 0.39, being similar to the ratio of total weight.

Therefore, we conclude that the causes of slow scallop growth in Abashiri Bay are the extremely low temperature in winter and temperature fluctuations from spring to summer. The magnitude of this fluctuation should vary every year. In the western part of the Okhotsk Sea coast, including Abashiri D, the winter temperature is low, but temperatures from spring to summer are stable at which time the scallops grow well.

## Spring-summer scallop growth

Figure 13 shows total wet weight of scallops from April to September for 3 years. The weight is the average of 30 to 2000 scallops obtained by harvesting operations in each year. They show various growth rates in this season, such as rapid increase in weight from April to May (Abashiri B 1982, Abashiri D 1983) and decrease in weight from May to June caused by spawning. However, it is clear that in Abashiri B 1982 and Abashiri C 1984 the scallops grew very slowly from July to September. This slow growth is probably caused by the rapid temperature fluctuation as mentioned above.

## **Conclusions**.

Overall results of sowing-culture from 1978 to 1985 are shown in Figure 14. The highest weight was 170 g in 1983 at Abashiri D. In the other grounds of Abashiri Bay, Abashiri A, B, and C, the weight fluctuated between 140 and 60 g. It is clear that there is no trend toward increase or decrease in weight. We conclude that these fluctuating growth rates reflect the environmental instability in Abashiri waters.

On the other hand, the recatch:release ratio increased year after year to about 40% in 1985. This indicates that there is no relationship between growth and survival. The decrease of predation by starfish may explain the trend of increase in the survival ratio through the years. It is well known that starfish are the main predator of scallops. In these sowingculture grounds, the removal of starfish has been carried out successively since 1978. It is believed that the numbers of starfish have gradually decreased and the survival ratios



Figure 13 Growth of 2- to 3-year-old scallops at Abashiri Stations B (• 1982), D (0 1983), and C (× 1984), April to September.



Figure 14 Yearly variations of total wet weight of 3-year-old scallops in September (O) and release:recatch ratio (•) in the Abashiri scallop grounds (A-D), 1980-85.

have increased year after year. Actually, a marked decrease in numbers of starfish from  $0.39/m^2$  (June 1980) to  $0.07/m^2$  (July 1985) was observed in Abashiri A.

In the present study, we determined that environmental instability was especially marked in Abashiri Bay, causing slow and fluctuating growth. However, continuous efforts to eliminate predators such as starfish and to collect large, healthy seed scallops and release them at both a suitable time and a rational population density have brought about an abundant harvest even in Abashiri Bay, though the weight of individual scallops is small.

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## Mantis Shrimp: Its Fishery and Biological Production

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Seikai National Fisheries Research Institute Kokubu-machi, Nagasaki 850, Japan The mantis shrimp, Oratosquilla oratoria (de Haan), is a stomatopoda crustacean inhabiting the sandy mud bottom in the coastal waters of temperate and subtropical regions of the northwest Pacific Ocean. In Japan, this shrimp has been caught in large quantities (~5000 tons per year) in the coastal waters of Tokyo Bay, Ise Bay, and Seta Island Sea, and used for food in "Sushi." The fishery in Tokyo Bay (the process from fishing to forwarding) is described in this paper as one example of the fishery for this shrimp. Also the process of biological production of adult mantis shrimp is described, which is important as the basis of the explanation of population dynamics of this shrimp.

## Fishery in Tokyo Bay \_

The mantis shrimp is one of the most important fishing targets in Tokyo Bay. The shrimp fishery is conducted with the aid of a Danish seine boat (Fig. 1) carrying a type of hand trawl. After trawling for about one hour, the net is hauled (Fig. 2) and the mantis shrimps are selected from the catch on deck (Fig. 3). Shrimps are transported live in holding tanks and are boiled soon after landing (Fig. 4). The carapace (cephalothorax) and both sides of the abdominal exoskeleton are cut with scissors (Fig. 5) and the shells are removed by hand (Fig. 6). The shucked shrimp, graded by size, are packed in plastic cases (Fig. 7) and forwarded (Fig. 8).

The fishermen engaged in the Tokyo Bay fishery have restricted themselves to the ratio of 2:1 days (i.e., 4 days operation per week) to ensure the shrimps' stable production and to preserve its price in the market by controlling the volume produced. Compared with other production areas, that of Tokyo Bay is relatively stable.

## Process of biological production .

In contrast to the Tokyo Bay fishery, the annual catch of mantis shrimp fluctuates widely among the prefectures. To maintain the catch at a certain level, two problems must be resolved: One is to develop techniques of resource management, and the other is to intensify efforts for artificial recruitment to the shrimp population. To resolve these problems, systematic ecological investigations of the natural populations and of the primary factors affecting population dynamics are needed. Until now, however, these kinds of studies have been rare.

A series of investigations was undertaken by the author to determine the biological production of adult mantis shrimp, based on the ecological aspects of reproduction, growth, and food consumption, and to clarify the distribution of energy from food intake to the growth of reproductive organs and other body parts. All shrimp used in this research were obtained from Mutsu Bay, Aomori Prefecture of northern Japan.



Figure 1 Danish seine boat carrying a type of hand trawl.

Figure 2 Net hauled after about one hour trawling.

Figure 3 Mantis shrimps selected from the catch on deck.

Figure 4 Mantis shrimps boiled soon after landing.

Figure 5 Cutting both sides of the abdominal exoskeleton with scissors.

Figure 6 Shucking the shells by hand.

Figure 7 Shucked shrimps packed in plastic cases.

Figure 8 Shrimps prior to forwarding.



 Figure 9

 Scheme of the reproductive cycles of the mantis shrimp. △ Recovering, ○ developing

 ③ prematuring, ● maturing, ○ spawning (female) and sperm-ejecting (male) period.

#### **Reproductive cycle**

The reproductive cycle of mantis shrimp was described by Yamazaki and Fuji (1980), summaried as follows.

The developmental processes of germ cells in both sexes were arbitrarily categorized into stages by histological techniques. On the basis of these stages of the development of germ cells, the maturation processes were classified into four stages in the ovary and five stages in the testis. The value of the gonad index, which is the ratio of the wet weight of gonadal tissues to the total body weight, is correlated with the gametogenetic development in both sexes.

The annual reproductive cycle in the female population of mantis shrimp was broadly classified into the following five periods, according to the course of seasonal variation of the gonad index and the duration of active appearance of each gonadal stage: 1) Recovering (Aug-Sept), 2) developing (Oct-Nov), 3) prematuring (Dec-Feb), 4) maturing (Mar-May), and 5) spawning (June-July). In similar procedures, the cyclical reproductive processes in the male population were divided as follows: 1) Recovering (July), 2) developing (Aug-Sept), 3) prematuring (Oct-Dec), 4) maturing (Jan-Apr), and 5) sperm-ejecting (May-June) (Fig. 9).

### **Growth pattern**

**Change in body length** Mantis shrimps were sampled monthly by bottom gill net (7.6 cm mesh) April 1976–September 1977 and May 1981–December 1982. From polymodal length-frequency distributions, several fitted normal curves were analyzed by the probability graph paper method of Harding (1949) (Fig. 10). And from these normal curves, the seasonal fluctuation in mean body length of each cohort was obtained. The rearing experiment for the observation of moulting was made September 1981–December 1982, and moulting was observed only August–October

(Table 1). From these results, adult growth in length appeared stepwise (Fig. 11). From three groups of pre- and post-moulting body length presented in Figure 11, the following equation is derived by means of the graphic method of Hiatt (1948).

$$L_{n+1} = 0.755L_n + 4.743 \tag{1}$$

where  $L_n$  and  $L_{n+1}$  are the linear dimensions of pre- and postmoulting, respectively.

The value of 0.755 shows that its growth pattern is of the retrogressive geometric type (Kurata 1960), and that the amount of increase at successive moultings decreases in proportion to the increase in initial premoulting body length.

**Changes in body weight and body component weight** (integument, muscle, hepatopancreas and gonad) Lengthweight regression equations were obtained monthly from the rearing experiment, and the slopes of these equations were unified to a value by statistical analysis (Table 2). From these equations and the body length derived from the equation (1), the seasonal body-weight change was derived (Fig. 12). Body weight (and body length) increases by a large increment after moulting, and there is a remarkable variation resulting from maturation and spawning in the female.

The regression equations of body weight-dry component weight were obtained from bimonthly dissections. Based on these equations, the seasonal changes in dry weight of body components were derived from any body length without dissections (Table 3). The striking seasonal variations of dry weight of body components reflect the accumulation and consumption of substances concerned with spawning and moulting in females. However, in males there are no large changes throughout the year except the increment at moulting.



### Figure 10

Histograms showing frequencies of body length and several fitted normal curves calculated by the probability graph-paper method. Broken lines indicate the flow of mean body length of each cohort.



Number	of	f mo		0		ivid	Fab Iuals 981-	an	nong				hrin	nps	rea	rec
		Month														
	S	0	N	D	J	F	М	Α	М	J	J	Α	S	0	N	I
Female	2	11	0	0	0	0	0	0	0	0	0	3	6	5	0	(
Male	3	13	0	0	0	0	0	0	0	0	0	3	8	7	0	(
Total	5	24	0	0	0	0	0	0	0	0	0	6	14	12	0	(

## Figure 11

Seasonal variation in modal length of the shrimp body for each sizegroup. Each mark indicates the mean body length of each cohort shown in Figure 10. Straight lines indicate the mean values of marks within each size-group. In Group I, mean values are 12.2 cm (pre-moulting) and 13.8 cm (post-moulting); in Group II, 14.0 cm and 15.6 cm; and in Group III, 15.9 cm and 16.6 cm, respectively.

Table 2Values of constant (a) in length-weight regressionequation, $W = aL^{3.068}$ , of the mantis shrimps reared.									
Date	Female	Male							
2 Nov	-1.935	-1.925							
16 Dec	-1.946	-1.938							
5 Jan	-1.940	-1.923							
2 Mar	-1.942	-1.917							
6 May	-1.934	-1.924							
9 June	-1.915	-1.933							
10 July	-1.955	-1.938							
17 Aug	-1.951	-1.929							
6 Sept	-1.935	-1.944							
5 Oct	-1.956	-1.943							
4 Nov	-1.947	-1.944							
4 Dec	-1.964	-1.954							



## Figure 12

Seasonal change in body weight calculated by substituting the lengthweight regression models shown in Table 2 for the body length derived from the equation  $L_{n+1} = 0.755L_n + 4.743$ , starting with 12.2 cm. Circles indicate female weight and triangles indicate male. Values at spawning and moulting are shown at 15 June and 15 September with open marks.

#### Table 3

Changes in dry weight of muscle, hepatopancreas, integument, exuviae, gonad, and spawn of mantis shrimp. Values of body length are derived from the equation,  $L_{n+1} = 0.755 L_n + 4.743$ , starting from 12.2 cm. Values of body weight are derived from body lengths and values of *a* from the equation,  $W = aL^{3.068}$  (table 2). Values of each body component are derived from body weight and the regression equations of body weight-dry component weight.

			Deter	Body weight	Dry weight (g)											
Size		Body length	Body w (g)	•			Fem	ale				Mal	le			
group	Date	(cm)	Female	Male	Muscle	Hepat.	Integ.	Exu.	Gonad	Spawn	Muscle	Hepat.	Integ.	Exu.		
	12 Dec	12.2	24.4	24.9	1.98	0.27	2.77	0.00	0.18	0.00	2.02	0.20	3.16	0.00		
	9 Feb	"	24.7	25.9	2.00	0.19	2.81	0.00	0.10	0.00	2.11	0.22	3.27	0.00		
	11 Apr	"	24.9	25.8	2.02	0.25	2.83	0.00	0.15	0.00	2.10	0.21	3.26	0.00		
	9 June	"	26.2	25.1	2.14	0.17	2.96	0.00	0.87	0.00	2.04	0.21	3.18	0.00		
Ι	15 June	"	23.6		1.90	0.15	2.69	0.00	0.05	1.10						
	10 Aug	"	24.1	25.2	1.95	0.12	2.74	0.00	0.07	0.00	2.05	0.21	3.19	0.00		
	6 Sept	"	25.0	24.5	2.03	0.21	2.84	0.00	0.04	0.00	1.99	0.20	3.11	0.00		
	15 Sept	14.0	34.4	37.4	2.89	0.33	1.59	1.85	0.07	0.00	3.17	0.36	1.71	2.00		
	13 Oct	"	36.5	37.4	3.09	0.23	4.04	0.00	0.05	0.00	3.17	0.36	4.61	0.00		
	22 Dec	"	36.8	38.0	3.11	0.48	4.07	0.00	0.39	0.00	3.23	0.37	4.68	0.00		
	12 Dec	14.0	37.3	38.0	3.16	0.49	4.12	0.00	0.40	0.00	3.23	0.37	4.68	0.00		
	9 Feb	"	37.6	39.5	3.19	0.34	4.15	0.00	0.23	0.00	3.37	0.39	4.85	0.00		
	11 Apr	"	37.9	39.3	3.22	0.45	4.18	0.00	0.32	0.00	3.35	0.39	4.83	0.00		
	9 June	"	39.9	38.3	3.41	0.32	4.39	0.00	1.92	0.00	3.26	0.37	4.72	0.00		
п	15 June	"	36.1		3.04	0.27	3.99	0.00	0.11	1.70						
	10 Aug	"	36.7	38.6	3.11	0.22	4.06	0.00	0.16	0.00	3.28	0.38	4.75	0.00		
	6 Sept	"	38.1	37.4	3.24	0.39	4.20	0.00	0.09	0.00	3.17	0.36	4.61	0.00		
	15 Sept	15.3	45.1	49.0	3.90	0.49	2.04	2.41	0.12	0.00	4.28	0.53	2.20	2.62		
	13 Oct	"	48.0	49.1	4.18	0.34	5.21	0.00	0.08	0.00	4.29	0.53	5.94	0.00		
	22 Dec	"	48.3	49.9	4.21	0.71	5.24	0.00	0.65	0.00	4.36	0.54	6.03	0.00		

## Table 3 (continued)

		Table 5 (continued)													
			Body w	eight					Dry we	eight (g)					
Size		Body length	(g)	0		Female Male									
group	Date	(cm)	Female	Male	Muscle	Hepat.	Integ.	Exu.	Gonad	Spawn	Muscle	Hepat.	Integ.	Exu.	
	12 Dec	15.3	48.9	49.8	4.27	0.72	5.30	0.00	0.66	0.00	4.35	0.54	6.02	0.00	
	9 Feb	"	49.3	51.9	4.31	0.50	5.34	0.00	0.38	0.00	4.56	0.57	6.26	0.00	
	11 Apr	"	49.8	51.6	4.35	0.67	5.39	0.00	0.54	0.00	4.53	0.57	6.23	0.00	
	9 June	"	52.4	50.3	4.61	0.46	5.65	0.00	3.19	0.00	4.40	0.55	6.08	0.00	
Ш	15 June	"	47.3		4.11	0.40	5.14	0.00	0.18	2.26					
	10 Aug	"	48.2	50.6	4.20	0.33	5.23	0.00	0.27	0.00	4.43	0.55	6.11	0.00	
	6 Sept	"	50.1	49.0	4.38	0.57	5.42	0.00	0.15	0.00	4.28	0.53	5.93	0.00	
	15 Sept	16.3	54.8	59.6	4.84	0.65	2.45	2.92	0.10	0.00	5.31	0.70	2.65	3.18	
	13 Oct	"	58.2	59.7	5.17	0.44	6.24	0.00	0.11	0.00	5.32	0.70	7.13	0.00	
	22 Dec	"	58.7	60.6	5.22	0.94	6.29	0.00	0.93	0.00	5.41	0.71	7.23	0.00	

## Food consumption

Food consumption of mantis shrimp was reported by Yamazaki (1985) and is summarized as follows.

Daily amounts of food eaten and feces excreted by the shrimp were measured during a period of about one year, December 1981–December 1982. Because crustaceans often appear in stomachs of wild shrimp, euphausids were used for food. Daily feeding rates are high August–October and are about five times as high as the lowest value January– March (Table 4). However, assimilation efficiencies are at the level of about 90% in almost all months.

## **Bioenergetics**

The process of individual production may be described using the following equation:

$$G + R = A = C - E$$

where G = growth, R = metabolic loss, A = assimilation, C = consumption, and E = egestion. Values of each parameter were calculated in the following manner:

**Growth** is presented as the amount of accumulated energy during rearing periods. The amounts of energy of each body component were obtained by bimonthly weight determinations of body components listed in Table 3 and their caloric values determined with a bomb calorie meter (Table 5), and the accumulated energy was obtained from the differences between these bimonthly determinations shown with the caloric unit (Table 6).

The muscle achieves a large accumulation of energy at moulting. Due to moulting, the integument also shows a rapid accumulation and consumption of energy during September and October. In females, there is a large accumulation and consumption of energy before and after spawning.

Daily a	mounts of	0	Table 4and assimilaof mantis	tion efficiencie	es in each size
		Food	Feces	Food	Assimilation
Size		eaten	excreted	assimilated	efficiency
group	Month		(mg dry we	ight)	(%)
	Dec	52.15	4.99	47.16	90.4
	Jan	33.50	11.05	22.45	67.0
	Feb	69.48	6.51	62.97	90.6
	Mar	91.60	13.20	78.40	85.6
	Apr	102.76	6.78	95.98	93.4
	May	139.09	16.38	122.71	88.2
I	June	116.08	5.32	110.76	95.4
	July	237.64	10.58	227.06	95.5
	Aug	269.71	13.05	256.66	95.2
	Sept	204.86	8.63	196.23	95.8
	Oct	147.51	13.50	134.01	90.8
	Nov	134.64	26.58	108.06	80.3
	Dec	90.19	6.09	84.10	93.2
	Dec	57.96	3.42	54.54	94.1
	Jan	40.12	1.88	38.24	95.3
	Feb	26.32	0.56	25.76	97.9
	Mar	120.20	8.55	111.65	92.9
	Apr	138.06	8.82	129.24	93.6
	May	158.65	14.06	144.59	91.1
п	June	197.46	3.09	194.37	98.4
	July	224.15	11.69	212.46	94.8
	Aug	226.71	7.82	218.89	96.6
	Sept	214.63	12.98	201.65	94.0
	Oct	313.72	26.38	287.34	91.6
	Nov	272.81	21.54	251.27	92.1
	Dec	88.19	3.15	85.04	96.4
	Dec	55.15	2.50	52.65	95.5
	Jan	25.24	1.70	23.54	93.3
	Feb	21.06	10.02	11.04	52.4
	Mar	54.65	9.98	44.67	81.7
	Apr	128.90	20.34	108.56	84.2
	May	171.11	8.52	162.59	95.0
Ш	June	190.17	10.69	179.48	94.4
	July	232.78	16.94	215.84	92.7
	Aug	365.99	34.78	331.21	90.5
	Sept	229.21	16.96	212.25	92.6
	Oct	176.22	17.87	158.35	89.9
	Nov	151.00	5.37	145.27	96.2
	Dec	44.18	3.84	40.34	91.3

			D. I.						Energy va	lue (kcal)				
Size		Body length	Body w (g)	0			Fem	ale				Mal	e	
group	Date	(cm)	Female	Male	Muscle	Hepat.	Integ.	Exu.	Gonad	Spawn	Muscle	Hepat.	Integ.	Exu.
	12 Dec	12.2	24.4	24.9	8.77	1.50	4.52	0.00	0.94	0.00	8.95	1.11	5.15	0.00
	9 Feb	"	24.7	25.9	8.86	1.06	4.58	0.00	0.52	0.00	9.35	1.22	5.33	0.00
	11 Apr	"	24.9	25.8	8.95	1.39	4.61	0.00	0.78	0.00	9.30	1.17	5.31	0.00
	9 June	11	26.2	25.1	9.48	0.95	4.82	0.00	4.55	0.00	9.04	1.17	5.18	0.00
Ι	15 June	"	23.6		8.42	0.83	4.38	0.00	0.26	6.17				
	10 Aug	"	24.1	25.2	8.64	0.67	4.47	0.00	0.37	0.00	9.08	1.17	5.20	0.00
	6 Sept	"	25.0	24.5	8.99	1.17	4.63	0.00	0.21	0.00	8.82	1.11	5.07	0.00
	15 Sept	14.0	34.4	37.4	12.80	1.83	2.59	3.02	0.37	0.00	14.04	2.00	2.79	3.26
	13 Oct	"	36.5	37.4	13.69	1.28	6.59	0.00	0.26	0.00	14.04	2.00	7.51	0.00
	22 Dec	"	36.8	38.0	13.78	2.67	6.63	0.00	2.04	0.00	14.31	2.06	7.63	0.00
	12 Dec	14.0	37.3	38.0	14.00	2.72	6.72	0.00	2.09	0.00	14.31	2.06	7.63	0.00
	9 Feb	"	37.6	39.5	14.13	1.89	6.76	0.00	1.20	0.00	14.93	2.17	7.91	0.00
	11 Apr	'	37.9	39.3	14.26	2.50	6.81	0.00	1.67	0.00	14.84	2.17	7.87	0.00
	9 June	"	39.9	38.3	15.11	1.78	7.16	0.00	10.04	0.00	14.44	2.06	7.69	0.00
II	15 June	'	36.1		13.47	1.50	6.50	0.00	0.58	9.54				
	10 Aug	"	36.7	38.6	13.78	1.22	6.62	0.00	0.84	0.00	14.53	2.11	7.74	0.00
	6 Sept	"	38.1	37.4	14.35	2.17	6.85	0.00	0.47	0.00	14.04	2.00	7.51	0.00
	15 Sept	15.3	45.1	49.0	17.28	2.72	3.33	3.93	0.63	0.00	18.96	2.95	3.59	4.27
	13 Oct	"	48.0	49.1	18.52	1.89	8.49	0.00	0.42	0.00	19.00	2.95	9.68	0.00
	22 Dec	"	48.3	49.9	18.65	3.95	8.54	0.00	3.40	0.00	19.31	3.00	9.83	0.00
	12 Dec	15.3	48.9	49.8	18.92	4.00	8.64	0.00	3.45	0.00	19.27	3.00	9.81	0.00
	9 Feb	"	49.3	51.9	19.09	2.78	8.70	0.00	1.99	0.00	20.20	3.17	10.20	0.00
	11 Apr	"	49.8	51.6	19.27	3.73	8.79	0.00	2.82	0.00	20.07	3.17	10.15	0.00
	9 June	"	52.4	50.3	20.42	2.56	9.21	0.00	16.68	0.00	19.49	3.06	9.91	0.00
ш	15 June	"	47.3		18.21	2.22	8.38	0.00	0.94	12.68				
	10 Aug	"	48.2	50.6	18.61	1.83	8.52	0.00	1.41	0.00	19.62	3.06	9.96	0.00
	6 Sept	"	50.1	49.0	19.40	3.17	8.83	0.00	0.78	0.00	18.96	2.95	9.67	0.00
	15 Sept	16.3	54.8	59.6	21.44	3.61	3.99	4.76	0.52	0.00	23.52	3.89	4.32	5.18
	13 Oct		58.2	59.7	22.90	2.45	10.17	0.00	0.58	0.00	23.57	3.89	11.62	0.00
	22 Dec		58.7	60.6	23.12	5.23	10.25	0.00	4.86	0.00	23.97	3.95	11.78	0.00

		Am	ount of al	6 Table 5, 6	expressed i	in kilocalo	ries.						
Size	Duration of rearing								Ma	e		Total	
group	(days)	Muscle	Hepat.	Integ.	Exu.	Gonad	Spawn	Muscle	Hepat.	Integ.	Exu.	Female	Male
	59 (Dec-Feb)	0.09	-0.44	0.06	0.00	-0.42	0.00	0.40	0.11	0.18	0.00	-0.71	0.69
	61 (Feb-Apr)	0.09	0.33	0.03	0.00	0.26	0.00	-0.05	-0.05	-0.02	0.00	0.71	-0.12
	59 (Apr-June)	0.53	-0.44	0.21	0.00	3.77	0.00	-0.26	0.00	-0.13	0.00	4.07	-0.39
I	6 (June-June) 56 (June-Aug)	-1.06 0.22	-0.12 -0.16	-0.44 0.09	0.00 0.00	-4.29 0.11	6.17 0.00	0.04	0.00	0.02	0.00	0.26 0.26	0.06
	27 (Aug-Sept)	0.35	0.50	0.16	0.00	-0.16	0.00	-0.26	-0.06	-0.13	0.00	0.85	-0.45
	9 (Sept-Sept)	3.81	0.66	-2.04	3.02	0.16	0.00	5.22	0.89	-2.28	3.26	5.61	7.09
	28 (Sept-Oct)	0.89	-0.55	4.00	0.00	-0.11	0.00	0.00	0.00	4.72	0.00	4.23	4.72
	70 (Oct-Dec)	0.09	1.39	0.04	0.00	1.78	0.00	0.27	0.06	0.12	0.00	3.30	0.45

Table 6 (continued)													
Size	Duration of rearing (days)	Female				Male			Total				
group		Muscle	Hepat.	Integ.	Exu.	Gonad	Spawn	Muscle	Hepat.	Integ.	Exu.	Female	Male
	59 (Dec-Feb)	0.13	-0.83	0.04	0.00	-0.89	0.00	0.62	0.11	0.28	0.00	-1.55	1.01
	61 (Feb-Apr)	0.13	0.61	0.05	0.00	0.47	0.00	-0.09	0.00	-0.04	0.00	1.26	-0.13
	59 (Apr-June)	0.85	-0.72	0.35	0.00	8.37	0.00	-0.40	-0.11	-0.18	0.00	8.85	-0.69
п	6 (June–June) 56 (June–Aug)	-1.64 0.31	-0.28 -0.28	-0.66 0.12	0.00 0.00	-9.46 0.26	9.54 0.00	0.09	0.05	0.05	0.00	-2.50 0.41	0.19
	27 (Aug-Sept)	0.57	0.95	0.23	0.00	-0.37	0.00	-0.49	-0.11	-0.23	0.00	1.38	-0.83
	9 (Sept-Sept)	2.93	0.55	-3.52	3.93	0.16	0.00	4.92	0.95	-3.92	4.27	4.05	6.22
	28 (Sept-Oct)	1.24	-0.83	5.16	0.00	-0.21	0.00	0.04	0.00	6.09	0.00	5.36	6.13
	70 (Oct-Dec)	0.13	2.06	0.05	0.00	2.98	0.00	0.31	0.05	0.15	0.00	5.22	0.51
	59 (Dec-Feb)	0.17	-1.22	0.06	0.00	-1.46	0.00	0.93	0.17	0.39	0.00	-2.45	1.49
	61 (Feb-Apr)	0.18	0.95	0.09	0.00	0.83	0.00	-0.13	0.00	-0.05	0.00	2.05	-0.18
ш	59 (Apr-June)	1.15	-1.17	0.42	0.00	13.86	0.00	-0.58	-0.11	-0.24	0.00	14.26	-0.93
	6 (June-June)	-2.21	-0.34	-0.83	0.00	-15.74	12.68	-0.13	-0.13 0.00	0.05	0.00	-6.44	0.18
	56 (June-Aug)	0.40	-0.39	0.14	0.00	0.47	0.00				0.00	0.62	0.10
	27 (Aug-Sept)	0.79	1.34	0.31	0.00	-0.63	0.00	0.66	-0.11	-0.29	0.00	1.81	-1.06
	9 (Sept-Sept)	2.04	0.44	-4.84	4.76	-0.26	0.00	3.56	0.94	-5.35	5.18	2.14	4.33
	28 (Sept-Oct)	1.46	-1.16	6.18	0.00	0.06	0.00	0.05	0.00	7.30	0.00	6.54	7.35
	70 (Oct-Dec)	0.22	2.78	0.08	0.00	4.28	0.00	0.40	0.06	0.16	0.00	7.36	0.62

**Metabolic loss** The amount of oxygen consumption in each individual housed in a plastic chamber was measured by the Winkler method for 7–9 shrimp of various sizes. A regression equation among oxygen consumption per unit time, water temperature, and body weight was obtained (Fig. 13). From this equation and monthly mean values of body weight and water temperature, monthly oxygen consumptions were calculated. The standard metabolism in terms of caloric value was determined by multiplying the oxygen consumption by 4.83 cal/mL $-O_2$  (Ivlev 1934).

The amount of metabolic loss was assumed as twice the standard metabolism from the literature (McLeese 1964, McFarland and Pickens 1965, Nelson et al. 1977a,b, Logan and Epifanio 1978) in which specific dynamic action and swimming were considered (Table 7).

The amounts of energy released for metabolic activity are in the range of  $0.46-0.76 \text{ kcal} \cdot \text{day}^{-1} \cdot \text{ind}^{-1}$  during June and October, and are under  $0.4 \text{ kcal} \cdot \text{day}^{-1} \cdot \text{ind}^{-1}$  during the winter period of inactive feeding, unrelated to differences in body size or sex.

Assimilation The equation for the amount of assimilation was G + R = A (Table 6).

**Consumption** In this paper, food consumption is calculated by *A*/assimilation effeciency. From the monthly values of daily ingestion the daily defecation rates obtained with the units of weight in Table 4 and the caloric values of euphausids (6.05 kcal  $\cdot$  g dry wt<sup>-1</sup>) and defecations (5.09 kcal  $\cdot$  g dry wt<sup>-1</sup>), the assimilation efficiency was calculated in terms of caloric value (Table 8). Further, the monthly values



Figure 13 Logarithmic plots of respiratory rate against body weight. ● 9.0°C; ○ 11.5°C; ▲ 15.0°C; △ 19.2°C. The base of logarithms in this equation is 10.

of assimilation divided by these efficiencies gave the bimonthly values of consumption (Table 7).

Seasonal variations of ingestion energy shown in Table 7 generally have the same tendencies as that of the daily amounts of food eaten shown in Table 4.

Group	Duration of rearing (days)	Metabolic loss (R)		Total growth (G)		Assimilation energy (A)		Ingestion (C)		Egestion (E)	
		Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
	59 (Dec-Feb)	17.40	17.56	-0.71	0.69	16.69	18.25	20.45	22.37	3.76	4.12
	61 (Feb-Apr)	17.10	17.30	0.71	-0.12	17,81	17.18	19.70	19.00	1.89	1.82
	59 (Apr-June)	22.78	22.78	4.07	-0.39	26.85	22.39	29.06	24.23	2.21	1.84
I	6 (June-June) 56 (June-Aug)	2.78 29.84	33.10	0.26 0.26	0.06	3.04 30.10	33.16	34.48	34.51	1.34	1.35
	27 (Aug-Sept)	16.22	16.30	0.85	-0.45	17.07	15.85	17.76	16.49	0.69	0.64
	9 (Sept-Sept)	5.58	5.64	5.61	7.09	11.19	12.73	11.60	13.19	0.41	0.46
	28 (Sept-Oct)	17.18	17.40	4.23	4.72	21.41	22.12	22.63	23.38	1.22	1.20
	70 (Oct-Dec)	34.04	34.30	3.30	0.45	37.34	34.75	41.91	39.11	4.57	4.30
	59 (Dec-Feb)	19.66	19.86	-1.55	1.01	18.11	20.87	18.86	21.74	0.75	0.8
	61 (Feb-Apr)	19.30	19.52	1.26	-0.13	20.56	19.39	21.55	20.32	0.99	0.93
	59 (Apr-June)	25.74	25.72	8.85	-0.69	34.59	25.03	36.76	26.60	2.17	1.57
	6 (June-June)	3.14	37.38	-2.50	0.19	0.64	37.57	35.86	38.77	1.11	1.20
Π	56 (June-Aug)	33.70		0.41		34.11		55.00		1.11	
	27 (Aug-Sept)	18.32	18.42	1.38	-0.83	19.70	17.59	20.39	18.21	0.69	0.62
	9 (Sept-Sept)	6.14	6.22	4.05	6.22	10.19	12.44	10.73	13.09	0.54	0.6
	28 (Sept-Oct)	18.60	18.88	5.36	6.13	23.96	25.01	25.49	26.61	1.63	1.60
	70 (Oct-Dec)	36.82	37.10	5.22	0.51	42.04	37.61	44.53	39.84	2.49	2.23
	59 (Dec-Feb)	21.24	21.46	-2.45	1.49	18.79	22.95	20.95	25.59	2.16	2.64
	61 (Feb-Apr)	20.88	21.12	2.05	-0.18	22.93	20.94	29.66	27.09	6.73	6.15
	59 (Apr-June)	27.86	27.80	14.26	-0.93	42.12	26.87	45.39	32.19	3.27	5.32
	6 (June–June)	3.40	40.42	-6.44	0.18	-3.04	40.60	36.13	43.15	2.13	2.55
III	56 (June-Aug)	36.42		0.62		37.04					
	27 (Aug-Sept)	19.82	19.90	1.81	-1.06	21.63	18.84	23.41	20.39	1.78	1.55
	9 (Sept-Sept)	6.58	6.64	2.14	4.33	8.72	10.97	9.30	11.70	0.58	0.73
	28 (Sept-Oct)	19.68	19.96	6.54	7.35	26.22	27.31	28.28	29.46	2.06	2.15
	70 (Oct-Dec)	38.94	39.26	7.36	0.62	46.30	39.88	49.20	42.38	2.90	2.50

Table 8           Assimilation efficiencies (%) of mantis shrimp size groups calculated in terms of caloric value.							
	Size group						
Month	I	п	ш				
Dec	91.9	95.0	96.2				
Jan	72.2	96.0	94.4				
Feb	92.1	98.2	60.0				
Mar	87.9	94.0	84.6				
Apr	94.4	94.6	86.7				
May	90.1	92.5	95.8				
June	96.1	98.7	95.3				
July	96.2	95.6	93.9				
Aug	96.0	97.1	92.0				
Sept	96.5	95.0	93.8				
Oct	92.3	92.9	91.5				
Nov	83.4	93.4	96.8				
Dec	94.3	97.0	92.7				

**Egestion** The equation for the amount of egestion was C - A = E (Table 7). Tables 6 and 7 present the seasonal variations of energy budgets per about a two-month period in terms of consumption, assimilation, accumulation, and egestion of energy. In the sum of these values, the energy budgets for the year are shown in Table 9.

In adult shrimp, a total of 190–240 kcal per year are ingested and about 90% is assimilated. Almost all the assimilated energy is lost by metabolism, and the remaining energy (19–26 kcal in females, about 12 kcal in males) is accumulated in each body component, of which 7–14 kcal is used for growth of the ovary and 85–90% is released from the body as reproductive substances at spawning. Gross growth efficiencies are indicated 9–11% in females and 5–6% in males.

Further, if quantitative changes in growth and food consumption of larva and young are investigated on the basis of the above methods, the life history of mantis shrimp will be clarified.

Table 9           Energy budget in the mantis shrimp.								
	Gro	up I	Grou	up II	Group III			
(Kcal/year)	Female	Male	Female	Male	Female	Male		
Food								
Food ingested	197.59	192.28	214.17	205.18	242.32	231.95		
Feces excreted	16.09	15.85	10.27	9.67	21.61	23.59		
Food assimilated	181.50	176.43	203.90	195.51	220.71	208.36		
Metabolic loss	162.92	164.38	181.42	183.10	194.82	196.56		
Growth								
Total	18.58	12.05	22.48	12.41	25.89	11.80		
Muscle	5.01	5.36	4.65	5.00	4.20	3.70		
Hepatopancreas	1.17	0.95	1.23	0.94	1.23	0.95		
Integument	2.11	2.48	1.82	2.20	1.61	1.97		
Exuviae	3.02	3.26	3.93	4.27	4.76	5.18		
Gonad	1.10	_	1.31	_	1.41	_		
Gametes ejected	6.17		9.54	—	12.68	—		

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## Growth and Survival of Artificial Abalone Seed Released in Shijiki Bay, Japan

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## ABSTRACT

The growth rate and survival rate of artificially produced young abalone, *Haliotis discus*, on the fishing ground was determined in order to develop a method for evaluating its ecological and biological characteristics. Daily growth ( $\mu$ m/day) decreased during the period with the high water temperature. The mean daily growth differed between the two stations. Survival rates decreased throughout the experiment, and the largest decrease occurred just after release.

Abalone is a major fishery of rocky coastal Japan, priced at about 5000 yen/kg. Annual catches from 1975 to 1984 were between 3900 and 5650 tons. Total world catch in 1982 was approximately 20,000 tons. Japanese consumption of abalones is estimated at about 9000 tons. Japan leads in abalone consumption and is the second leading producer, after Australia (Uki 1985). The catch in Japan consists primarily of four species: Haliotis discus hannai occurs in the north, while the other species, H. discus, H. gigantea, and H. sieboldii, range from the central to the southern regions. Propagation of abalones through fishery management and protective breeding has had a long history in Japan. Recently, sea farming techniques of releasing hatchery seed have been developed. The number released in 1983 amounted to 17.5 million, composed of 64% H. discus hannai and 34% H. discus. The shell length of seeds at release was approximately 30 mm.

In many cases, however, it is not clear whether the releasing of seeds results in increased catch. The growth rate and survival rate of artificially produced young abalone, *H. discus*, on the fishing ground was determined in order to develop a method for evaluating its ecological and biological characteristics.

## Materials and methods -

Two experimental stations, A and B, were located in Skijiki Bay, Hirado island, in southern Japan (Fig. 1). The duration of the experiment was 20 March-25 September 1986.



Figure 1 Location of experimental stations, Shijiki Bay, southern Japan.



Figure 2 Distribution of shell length of abalone seed at release and recapture.

Station A was a pile of stones surrounded by sand at a depth of about 7 meters. It had some sargassum and other small seaweeds. The sargassum decreased after July, in the typical seasonal pattern. Some natural juvenile abalones lived in Station A. The area of the rock pile was about 150 m<sup>2</sup> and the height was about 1.5 m. Station A was selected because the surrounding sand would prevent dispersion of the planted abalones. Station B was on a rocky coast with much seaweed at a depth of 1–5 meters. The seawood was mostly sea oak, *Eisenia bicyclis*. Station B was considered a suitable environment for abalones and was selected to compare its growth rate with Station A.

The abalones, all *H. discus*, used in this study were obtained from Yamaguchi Prefecture (Group Y) and Miyagi Prefecture (Group M). Initial mean shell lengths were 31.5 mm and 29.2 mm, respectively. The numbers released at Station A were 996 of Group Y and 985 of Group M. Numbers released at Station B were 75 of Group Y and 315 of Group M. Individuals were distinguished by the color and number of a tag attached to the shell by adhesive.

Seeds were released by SCUBA divers on 20 March and sampled four times: 23 April, 27 May, 22 August, and 25



Figure 3 Seasonal changes in daily growth of abalone seed.

September. Samples were collected by two or three SCUBA divers for two hours each time. Color and number of the tags, shell length and body weight were determined. Measured abalones were taken to their former station and released carefully, but randomly, in order to obtain an accurate analysis.

Growth rate was indicated as daily growth ( $\mu$ m/day), calculated by dividing the increase of shell length by the days between release and recapture. This calculation used only the data of individuals recaptured in consecutive samplings. Survival rate was calculated from the total number released initially and the total number in the population estimated at each sampling. Total number in the population was estimated by Jolly-Seber's multiple recapture method.

## Results

The numbers of abalone recaptured at Station A were 217 on 23 April, 156 on 27 May, 44 on 22 August, and 20 on 25 September. Distribution of shell length of all individuals measured at initial release was compared with that of the recaptured individuals for groups Y and M (Fig. 2). In Group Y, both distributions are similar; however, in Group M, in the distribution of recaptured individuals, the smallest ones were virtually absent.
Table 1       Population of artificial abalone, H. discus, estimated by Jolly-Seber process.							
	No	N. I. I	No. recaptured				Estimated annulation
Time at release ( <i>i</i> ) Time at recapture ( <i>j</i> )	No. captured No. relea ni Si	No. released Si	<i>i</i> =1	2	3	4	Estimated population Ni
1	1981	1981	_				_
2	212	208	212				755
3	153	152	110	43			552
4	42	42	21	7	14		210
5	20	19	10	4	2	4	_



Figure 4 Seasonal changes in survival rate of abalone seed.

Daily growth of every group at each station decreased throughout the experiment (Fig. 3). Especially during 22 August-25 September, the period with the highest water temperature, there was no sign of increase in shell length at Station A. Also, the mean daily growth of Group M at Station A was always larger than that of Group Y, except for the period 22 August-25 September. In comparison, Station B had much more daily growth in Group M.

In the Jolly-Seber model,  $m_{hi}$ , number caught in the *i*th sample last captured in the *h*th sample, were recorded in Table 1. Total number in the population was calculated from that table, estimated by a personal computer program (Kato 1978).

Survival rates of groups Y and M decreased throughout the experiment, and the largest decrease occurred just after release (Fig. 4).

## **Discussion** .

Apparently, artificial seed varies in biological character when produced by different hatcheries. In this experiment, it was determined that daily growth and size distribution of recaptured individuals differed between groups Y and M. To go further in this field, it will be necessary to study the relationship between ecological character and production conditions.

The difference in daily growth between the two stations was experimentally confirmed. Although the growth rate is known to be altered by living conditions (Uki 1981, Inoue et al. 1986), the present experiment can be useful as a method for selecting a sea farming area. For that purpose it is necessary to obtain further data on growth rate and its seasonal variation in young abalone under natural conditions.

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## Copepod Swarms Observed by SCUBA Diving in a Small Inlet of Kyushu, Japan<sup>1</sup>

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### ABSTRACT

Swarms of copepods were observed by SCUBA diving in Shijiki Bay, southwest of Hirado Island, western Kyushu, Japan, during early spring to midsummer 1984–85. Copepods were densely distributed just above the sea bottom in the daytime and formed various features of swarm according to the configuration of sea bottom and season. Seven swarming copepod species, Acartia omorii, A. steueri, A. sinjiensis, Oithona oculata, O, davisae, Tortanus longipes, and T. rubidus, were identified. Each swarm consisted of many copepodite stages, sometimes of a single species but also of a mixture of several species. Species, stage and sex composition, shape of swarm, and swarming location varied seasonally. The swarm maintained a stationary spatial position when the water current was weak. Swarming of Acartia and Oithona may serve as an important food for some juvenile demersal fishes during their early demersal stages. In Shijiki Bay, pelagic larvae of red sea bream migrate into the Bay from offshore waters during April to May and become demersal. In the Bay, stomachs of demersal juveniles are filled with *Acartia omorii* and *A. steueri* (Tanaka 1985). These copepods, important food organisms for fish in a neritic society, have swarming characteristics.

Copepod swarms have been observed in dense crowds at specific spots in the neritic waters of the tropical, subtropical, temperate regions, and freshwater lakes of the Arctic as well. Many species in the genera *Oithona*, *Acartia*, *Centropages*, and *Labidocera* appear in swarming communities in neritic waters (Emery 1968, Hamner and Carleton 1979, Omori and Hamner 1982). Swarms of *Heterocope septentrionalis* and *Diaptomus tyrrelli* also occur in freshwater lakes (Hebert et al. 1980, Byron et al. 1983). Ueda et al. (1983) reported swarms of the copepod genera *Acartia*, *Oithona*, and *Labidocera* in the coastal waters of Japan.

Copepod swarms on the sea bottom must play a major role in the food strategy of juveniles of demersal fishes. In Shijiki Bay, copepod swarms were observed fragmentarily in 1978 and 1980 by Ueda et al. (1983). Therefore, in the Bay, the author has intensely investigated copepod swarms to learn the seasonal changes in distribution, locality, and size of swarms in the various areas.

### **Copepod** swarms

Copepod swarms were observed and collected at Shijiki Bay, southwestern Hirado Island, western Kyushu, Japan, from May to August 1984 and 1985 (Fig. 1). Repeated obser-



#### Figure 1

<sup>1</sup>Contribution No. 439 of the Seikai National Fisheries Research Institute.

Location of stations in Shijiki Bay, southwest of Hirado I., western Kyushu, Japan. Stations with slanted bar indicate where rope line was set.

Table 1       Copepod swarms observed by SCUBA diving in Shijiki Bay.				
Species	Shape and size of swarm	Depth (m)	Swarming location	
Acartia omorii	Continuous flat swarm, 5-30 cm thick	10-27	Over flat sandy bottom	
	Irregular balls, 10-30 cm diameter	10-24	Over flat sandy and gravelly bottom	
A. omorii + A. steueri	Continuous flat swarm, 5-30 cm thick	10-18	Over flat sandy bottom	
	Irregular balls, 10-50 cm diameter	10-13	Over flat sandy bottom	
	Irregular balls, 10-50 cm diameter	7	Over and throughout entire Zostera bed	
	Irregular balls, 10 cm-3 m diameter	2-7	Over rocky shore, around algal bed	
A. steueri	Irregular balls, 10 cm-1 m diameter	2-7	Over and throughout entire Zostera bed	
A. steueri + Oithona davisae	Irregular balls, 30-50 cm diameter	3	Edge of Zostera bed	
O. oculata	Irregular balls, 10-30 cm diameter	2-7	Inside Zostera bed, over rocky shore and gravelly bottom	
O. oculata + A. sinjiensis	Ball, 10 cm diameter	4	Edge of Zostera bed	
Tortanus longipes + T. rubidus	Column, 20 cm diameter, 50 cm height	2	Edge of Zostera bed	



#### Figure 2

Schematic illustrations of copepod swarming relative to bottom topography and materials in Shijiki Bay. a) Sandy bottom, b) Zostera marina bed, c) shoal of small rocks with brown algae, d) shoal of large rocks with brown algae.

were made by SCUBA diving along a 50-m long graduated nylon rope set on sandy bottom and eelgrass (*Zostera marina*) bed, 4–12 m in depth (L stations, Fig. 1). Observations were also made occasionally at gravelly and sandy bottom, 6–35 m in depth (S stations, Fig. 1). Swarming copepods were collected with a Van Dorn water sampler (6 L) or plastic suction bottle (3 L) like a syringe, and a hand-operated plankton net (50  $\mu$ m mesh opening).

A total of seven species of three genera were identified from swarming copepod communities; three species of *Acartia*, two of *Oithona*, and two of *Tortanus* (Table 1). A swarm sometimes consisted of a single species or genus and sometimes not. Species, stage, sex composition and shape of swarm, and swarming location varied seasonally.



Figure 3 (a) Seasonal changes in number of *Acartia steueri* and *A. omorii*, and percentages of (b) adults and (c) females in each species of *Acartia* collected by horizontal tow with plankton net at flat sandy bottom of inner part of Shijiki Bay, 12 May-13 July 1984.

Acartia omorii and A. steueri formed continuous flat swarms usually 5–30 cm thick, just above the flat sandy bottom in shallow waters 10–27 m depth (Stn. L-1, 2, S-1, 3, 5, 7, 8; Fig. 2a) from spring to early summer. Thickness and density of copepod swarms varied from place to place, and may be affected by the configuration of sea bottom and current strength. In the Bay, copepods sometimes formed a small ball or disk-shaped swarm. The sizes of swarms ranged from 10 cm to a few meters in diameter, but boundaries between swarm and background were not always clearly defined.

Copepod swarms were often observed in the lee of objects on the bottom such as clumps of living or dead algae, rocks, and trash. Swarm organisms usually held the same spatial position and swimming pattern against a moderate current. But when the current was stronger than about 5 cm/s, copepods were carried away from their initial position by the current.

Copepods swarm upward with a spiral movement and the swarm dispersed throughout the water column immediately after sunset. After sunrise the next morning, they swam downward and formed a swarm on the sea bottom again.



#### **Figure 4**

(a) Seasonal changes in number of Acartia steueri and A. omorii, and percentages of (b) adults and (c) females in A. steueri collected by horizontal tow with plankton net at eelgrass (Zostera marina) bed of inner part of Shijiki Bay, 12 May-13 July 1984.

A. steueri and A. omorii in swarms on flat sandy bottom were collected by horizontal tow of a plankton net. A number of organisms, including all developmental stages of adults, copepodites and nauplii, ranged from 8 to 190 per liter (Fig. 3a). This value is much higher than that collected by an ordinary plankton-net vertical haul. The ratio of adults (CVI) to copepodites (CII to CVI) for the above two species varied, but the mean was about 60 percent (Fig. 3b). Females of both species comprised about half of the adults (Fig. 3c).

At eelgrass (*Zostera marina*) beds with a range of 2–7 m in depth (Stn. L-3, 4, 7, 9, 10, S-1; Fig. 2b), dense swarms of *A. omorii* and *A. steueri* were observed during the observational period from March to July. Copepods swarmed within and near the eelgrass bed from the roots up to above the grass blades. Density of organisms, consisting of adults, copepodites, and nauplii, collected by the horizontal net towing inside the swarm ranged between 14 and 342 per liter (Fig. 4a). In *Acartia* swarms, adults were abuandant and females far outnumbered males, particularly in July (Fig. 4b, c). *Acartia* often swarmed in high densities along the edges of *Zostera* beds.

In the lee of rocks and brown algae, *Ecklonia stolonifera*, on a shoal (Stn. S-2, 4; Fig. 2c) 15–20 m deep in the central part of the Bay, *A. omorii* formed ball-shaped swarms with a diameter of 10–30 cm. On the other hand, in shoals near shore, 2–7 m deep in the inner part of the Bay, *A. omorii* and *A. steueri* formed large dense swarms in the lee of rocks and beds of brown algae (*E. stolonifera*, *Eisenia bicyclis*, and *Sargassum* spp.) (Stn. S-6, L-5, 6; Fig. 2d). These swarms were diverted right and left by waves. When disturbed by divers, they quickly resumed their initial position.

In summer, Oithona oculata formed swarms at a rocky reef and a Zostera bed in the inner part of the Bay (Stn. S-6, L-4, 9, 10). The Oithona swarm was ball-shaped with a diameter of 10 cm-1 m. Density of the O. oculata swarm was higher than that of Acartia, ranging from 490 to 6490 individuals per liter. A small ball-shaped swarm, consisting of O. oculata and Acartia sinjiensis, and large swarms, consisting of Oithona davisae and A. steueri, were also formed at the edges of the Zostera bed.

A small cylindrical swarm of *Tortanus longipes* and *T. rubidus* was observed at the edge of the *Zostera* bed. In the swarm, organisms moved in a swirl and maintained a cylindrical configuration.

# Swarming processes and functions of copepod swarms

In Shijiki Bay, swarms consisted of many copepodite stages, sometimes of a single species but also of a mixture of several species. The density of a swarm was much higher than that collected by ordinary vertical hauls of plankton net. Swarming during the day, copepodites of *Acartia* spp. and *Oithona* spp. aggregated to a density several hundred times denser than the mean density. However, the density changed both seasonally and daily, probably caused by seasonal population change and weather or tidal current conditions.

Present results suggest that copepod swarming is closely related to the microbottom topography and some materials on the bottom. The author also observed a copepod swarm on artificial materials, e.g., a gill net for crab fishing set on sandy bottom. Furthermore, the author could induce copepood swarms by field experiments with plastic boxes of different sizes (Kimoto unpubl.). Two processes of swarming by copepods were suggested: First is the increasing copepod density near-bottom by day during their diurnal vertical migration; second is that copepods swimming near the bottom are concentrated and form a much denser swarm in the lee of materials such as clumps of living or dead algae, rocks, and trash. The above copepods are probably carried into the lee by moderate currents. The spatial position of a copepod swarm is probably maintained by the wake formed behind the materials on the bottom. This interpretation was made by Kakimoto et al. (1983) who observed copepod (A. clausi = A. omorii) and mysid (Proneomysis fasca) swarms at natural and artificial reefs of the coastal area of Niigata Prefecture, Japan Sea. Copepods gathered at a certain density are probably able to recognize other individuals by chemical (Katona 1973), optic, and physical stimuli and fulfill some organic functions.

Adaptive functions of copepod swarms were suggested by previous authors (Hamner and Carleton 1979, Omori and Hamner 1982, Ueda et al. 1983). Hamner and Carleton (1979) suggested four adaptive functions: Protection from predators, facilitating breeding, maintaining a favorable position to feed on coral mucus, and restricting dispersion by currents. They suggested that, although protection from predators was the most common and important adaptive explanation for copepod swarming, all these adaptive functions were responsible for denser populations on coral reefs.

The importance of these adaptive functions may vary among species. Reduction of dispersion by currents appears to be of major importance for the swarms in maintaining populations of copepods. On the other hand, swarming probably poses a contradiction: When swarming organisms are distributed at the same space and time as demersal fishes, copepods tend to be eaten by fishes. Predation of A. steueri and A. omorii by demersal juveniles of red sea bream was observed in the Bay (Tanaka 1985). This suggests that the juveniles feed effectively on swarming Acartia, and, therefore, that copepod swarms are important in the feeding strategy of some fishes during their early demersal life. Copepod swarms could be induced by certain artificial structures constructed on the sea bottom; thus it may be possible to design a new nurseryground for juvenile fishes in the future.

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## Feeding Ecology of Young Red Sea Bream in Shijiki Bay

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## ABSTRACT

Feeding ecology of red sea bream in Shijiki Bay is explained especially with (1) changes in diet with growth, (2) predatorprev interactions between young red sea bream and gammaridean amphipods, and (3) feeding relationships between young red sea bream and other fish species. Juvenile and young red sea bream feed mainly on calanoid copepods and gammaridean amphipods, respectively, in the sandy bottom area of the inner part of the bay. They feed on calanoids when the calanoid swarm is formed and on gammarids when the gammarid density is highest. Crimson sea bream and stripedfin goatfish also change their main food from calanoids to gammarids as they grow in the sandy bottom area of the inner part of the bay. However, the peak month of feeding on calanoids and gammarids by these two fishes does not coincide with that of occurrence of each prey item in the field. Furthermore, young red sea bream always feed mainly on gammarids, independent of coexistence with hairychin goby; hairychin goby shift their main food from gammarids to mysids in the presence of red sea bream. These facts demonstrate that young red sea bream have an advantage over other fish species in feeding relationships.

Gammarids are the most important prey for young red sea bream; however, this fish cannot feed on all gammarid species in the field. Young red sea bream can add gammarids to their diet when gammarids become epibenthic. This fact points out the importance of discriminating the "true" prey species by species identification of prey organisms.

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Red sea bream Pagrus major is one of the most important demersal fishes for coastal fisheries in Japan because of its high landings and high market price. Since the 200-mile fishing jurisdiction was established, exploitation of the ocean potential around Japan became more imperative to meet the increasing demand for fish and shellfish of high quality. Recently, projects of stock enhancement have been promoted for the red sea bream through releasing operations, because mass production of juveniles has been established for this species in hatcheries. However, these releasing operations do not appear to have always resulted in increases to red sea bream stocks. There are two main keys to success of these projects: One is the qualitative evaluation of artificially propagated fish, and the other is accurate estimation of carrying capacity. For the latter, it is most important to understand fish biology and ecology.

A series of investigations on the ecology of 0-age red sea bream has been carried out since 1975 in Shijiki Bay to provide a biological basis for stock enhancement. The "Shijiki Project" deals with the early life history of red sea bream, fish communities, oceanographic conditions, primary and secondary production, experimental release of artificially propagated young red sea bream, etc.

In the present paper I explain the living modes of red sea bream, especially feeding ecology of the young. I also describe interspecific relationships between young red sea bream and other fish species.

## Living modes of red sea bream in Shijiki Bay \_\_\_\_\_

## **Environmental features**

Shijiki Bay, about 10 km<sup>2</sup> in area, is located at the southern end of Hirado Island in Nagasaki Prefecture, northwestern Kyushu (Fig. 1). The bottom consists mostly of well-sorted fine sand. *Zostera marina* grows in the inner part of the bay where the sandy bottom area is shallower than 7 m; *Sargassum* spp. grow in the reef area.

The flow pattern in Shijiki Bay indicates that the bay can be further subdivided. Two imaginary lines, one traced between Megasaki and Nagatenohana and another between Shiomibana and Iidabana, divide the bay into three parts: (1) the mouth is characterized by offshore waters; (2) the interior is characterized by proper embayed waters; and (3) the central area is characterized by a mixture of these two water masses (Tamai 1980). These divisions are also suggested by other environmental factors (Hamada 1980, Kiso 1980a, Sudo et al. 1983). Moreover, these divisions agree with the distribution of zooplankton fauna (Ueda 1980), macrobenthos fauna (Azuma and Jinno 1980) and fish fauna (Nakabo 1980).



Figure 1 Map of Shijiki Bay, Japan (depth contours in meters).

Shijiki Bay is essentially open because of the strong influence of the Tsuchima Current and the absence of rivers flowing into it. However, in the interior, detritus from seagrass accumulates on the bottom and is decomposed by bacteria under aerobic conditions (Sudo et al. 1983). The abundant detritus supports high productivity of benthic crustaceans, including gammaridean amphipods, and forms a healthy and fertile nursery ground for fishes in the inner part of the bay.

#### Growth and migration

The migration pattern of red sea bream in Shijiki Bay is closely related to the environmental structure of the bay (Fig. 2). Pelagic larvae hatch during March-May in offshore spawning grounds. They are transported by tidal currents and trapped by a circular current at the mouth of the bay. When a little larger than 10 mm in total length, larvae metamorphose into pelagic juveniles. These juveniles begin to migrate into the bay beyond the boundary between the outer and inner water mass. After immigration into the bay, juveniles become demersal at 12-15 mm total length in late May (Tanaka 1980, 1985).

Demersal juveniles feed on calanoid copepods swarming near the bottom, and gradually concentrate in the sandy bottom area of the interior. There, they feed heavily on gammaridean amphipods and grow at the rate of 0.7 mm per day. In June they reach the young stage, and in August they grow to 70–90 mm fork length and begin to extend their habitat toward the central area. While extending their habitat, mysids and other food items are added to their diet. The majority emigrate outside of the bay for wintering in September, although some remain in the bay until the next August (Azeta et al. 1980, Sudo et al. 1983).



Figure 2 Growth and migration pattern of 0-age red sea bream in Shijiki Bay (Sudo and Azeta 1986).

Feeding habits of young red sea bream .

## Dietary changes with growth

0-age red sea bream in Shijiki Bay change their main food with growth in the following order as shown in Figure 3 (Kiso 1980b): calanoid copepods, gammaridean amphipods, mysids. Demersal juveniles occurring in the sandy bottom area of the interior in late May feed mainly on calanoid copepods swarming near the bottom (Tanaka 1985). From June through August, when juveniles and young are most dense there, they feed heavily on gammaridean amphipods. These changes in diet appear to be related closely not only to prey size but also to prey density. Red sea bream feed on calanoid copepods when the calanoid swarm is formed and on gammaridean amphipods when the gammarid density is highest (Fig. 4). Furthermore, young red sea bream are densely distributed at sites where gammaridean amphipods are abundant (Azeta et al. 1980, Sudo et al. 1983). These facts emphasize that gammaridean amphipods are the most important prey for young red sea bream.

## **Predator-prey relationships**

Sudo et al. (1987) explained diel changes in predator-prey relationships between red sea bream and gammaridean amphipods. In Shijiki Bay, over 100 species of gammarids have been collected, and about 60 species of these occurred in the



Figure 3 Changes in diet of 0-age red sea bream with growth (Kiso 1980b).

sandy bottom area of the interior. However, about 50% of gammarid species in this area occurred in stomachs of young red sea bream. Moreover, seven species each surpassed 10% of the gammarids consumed: *Byblis japonicus*, *Synchelidium miraculum lenorostralum*, *Paradexamine marlie*, *Aoroides columbiae*, *Melita denticulata*, *Gitanopsis longus*, and *Tiron* sp. The proportion of the sum of these seven species to total gammarids in the stomachs ranged from 61.8% to 94.7% with diel time. Of these seven, *Byblis japonicus* was the most important prey species, because it was the most frequently consumed and largest in body length. *Synchelidium miraculum lenorostralum*, *Paradeximine marlie*, and *Melita denticulata* were also important prey species, because of their high frequency of occurrence and their large body size.

Comparing relative abundance of gammarid species in the stomachs with that in the field, individual gammarid species were not consumed relative to their abundances in the field. This difference between gammarid composition in the stomachs and in the field is believed to be due to the difference in availability of gammarid species. Stoner (1979) pointed out that amphipod selection by the pinfish Lagodon rhomboides was related most closely to the microhabitat of amphipod species, and important prey species were all epifaunal types. In Shijiki Bay, the patterns of gammarid selection by young red sea bream also correlated with the microhabitat of gammarid species. Epifaunal (e.g., Paradexamine marlie) and shallow burrowing types (e.g., Synchelidium miraculum lenorostralum) both were positively selected as prey, although the degree of selectivity for epifaunal types was higher than that for shallow burrowing



Monthly changes in density of gammaridean amphipods (mean  $\pm 1$  SD) in the sandy bottom area of the interior of Shijiki Bay April 1983– February 1984. Shaded zone indicates observed copepod swarm.

types. Infaunal tube-dwelling types (e.g., *Byblis japonicus*) were positively or negatively selected with diel time. Deep burrowing types (e.g., *Harpiniopsis vadiculus*, *Urothoe* sp. B, and *Urothoe* sp. C) were negatively selected or hardly consumed (Fig. 5). These results indicate that the availability of gammarid species increases with the decrease of gammarid living-depth in the sediment. This finding is consistent with my underwater observations that young red sea bream, a visual feeder, normally swim off the bottom and peck at prey organisms only when recognizing them.

However, there were diel changes in the pattern of predation on gammarid species by young red sea bream. The intensity of predation on Byblis japonicus was low about noon but increased remarkably at dusk and dawn, whereas that on Synchelidium miraculum lenorostralum and Paradexamine marlie increased about noon. This diel dietary shift is caused by diel vertical movements of gammarids, because vertical movements change their microhabitat and consequently influence their availability. Byblis japonicus, the most dominant species of gammarids in the field, lives in the tube in daytime; however, this species comes to the bottom surface in large numbers from dusk to dawn. On the other hand, Synchelidium miraculum lenorostralum digs in the superficial bottom sand exposing its dorsal part in the daytime, but swims up to the water column near the bottom in large numbers at night; Paradeximine marlie lives on the bottom surface in the daytime but swims up the water column to near surface in large numbers at night. Young red sea bream cannot feed fully on Byblis japonicus in the daytime, when this gammarid is in the tube; however, they can feed heavily on Byblis japonicus at dusk and dawn when it is on the bottom



#### Figure 5

Diel changes in microhabitat composition of gammarids in stomachs of young red sea bream (left) and in the field (right): EF, epifauna; SB, shallow burrower; DB, deep burrower; IT, infaunal tube dweller (Sudo et al. 1987).

surface or in the water column near bottom in large numbers (young red sea bream cease to feed after dark). On the other hand, they can feed on Synchelidium miraculum lenorostralum and Paradexamine marlie in the daytime, when both gammarids live on the bottom surface (Fig. 6). Thus, the diel dietary shift of young red sea bream does not contradict the thesis that epibenthic gammarids are most available. However, once gammarids come to the bottom surface, their abundance, body size, and moving speed (swimming or crawling) appear to become major factors influencing prey selectivity. In fact, Byblis japonicus, which was more abundant, larger in body length, and slower in movement (judging from its body shape and type of appendages) (Bousfield 1973), was subjected to heavier predation by young red sea bream than the other two gammarid species when it came to the bottom surface.

## Interspecific relationships

Sudo and Azeta (1986) described the interspecific relationships of young red sea bream to other fish species by comparing their niches. Here, in particular, I examine the feeding relationships among fishes. The niche has three main dimensions: time, habitat, and food (e.g., Pianka 1974, Christiansen and Fenchel 1977). Thus, the minor habitat among fishes is compared first and the fish species coexistent with red sea bream are picked out. Then their seasonal occurrence patterns and food habits are compared.

According to habitat analysis of demersal fishes with the index of interspecific overlapping (Cd) of Morisita (1959), fishes in the interior were divided into three groups: (1)



#### Figure 6

Diel predation patterns on three gammarid species heavily consumed by young red sea bream (upper), and diel vertical movement patterns of each gammarid species (lower). Black bars represent hours of darkness. Numerals indicate number of each gammarid species collected by one horizontal tow of larval net in water column (Sudo et al. 1987).

sandy bottom; (2) rocky bottom; and (3) eelgrass zone. Crimson sea bream (*Evynnis japonica*), stripedfin goatfish (*Upeneus bensasi*), and hairychin goby (*Sagamia geneionema*) as well as red sea bream belong to the sandy bottom group. Thus, seasonal occurrence patterns of these three species in the sandy bottom area of the interior are compared with that of red sea bream. Crimson sea bream and stripedfin goatfish



Figure 7 Changes in diet overlap index between red sea bream and hairychin goby in the sandy bottom area of the interior of Shijiki Bay (Sudo and Azeta 1986).

differ from red sea bream in the peak month of occurrence (crimson sea bream, mid-May; red sea bream, mid-June to mid-July; stripedfin goatfish, mid-August). On the other hand, hairychin goby overlap with red sea bream in both minor habitat and seasonal occurrence pattern. Moreover, these two species rank high in number among demersal fishes every year. Thus, the food habits of hairychin goby are compared with those of red sea bream.

Hairychin goby occurring in the sandy bottom area of the interior in late May feed mainly on calanoid copepods swarming near bottom. They then feed mainly on mysids from June through July; in August the majority migrate to the eelgrass zone in the interior (Matsumiya et al. 1980). The diet overlap index ( $\alpha$ ) of Schoener (1970) between red sea bream and hairychin goby is highest in late May when both are few in number, because both species feed on the same calanoid copepods. In June, when both increase in number, however, diet overlapping becomes insignificant, and the diet overlap index is lowest at their peaks of abundance in mid-June (Fig. 7). This is because red sea bream feed mainly on gammarids whereas hairychin goby feed mainly on mysids.

Figure 8 shows electivity indices (Ivlev 1961) of gammarids and mysids consumed by red sea bream and hairychin goby plotted against prey supply indices (prey supply index = biomass of each prey/total number of two fish species). As is evident from Figure 8, red sea bream prefer gammarids whereas hairychin goby prefer mysids, at sites where the two fish species coexist. On the other hand, at sites where the two fish species do not coexist, hairychin goby also prefer to feed on gammarids.

The relation between the electivity index and the prey supply index in Figure 8 demonstrates that red sea bream select gammarids more strongly with the increase of gammarid supply; however, there is no correlation between mysid selection by hairychin goby and mysid supply although mysids are the main food for hairychin goby. Moreover, hairychin goby begin to select gammarids with the increase



#### Figure 8

Ivlev's electivity indices of gammarids (top) and mysids (bottom) consumed by red sea bream (closed circle) and hairychin goby (open circle) plotted against prey supply indices. Prey supply index = Biomass of each two prey/total number of two fish species. On the left: electivity indices at sites where the two fish species coexist; on the right: electivity indices at sites where the two fish species do not coexist (Sudo and Azeta 1986, modified from Azuma et al. 1983).

in gammarid supply, independent of the coexistence with red sea bream.

These results suggest that young red sea bream always feed mainly on gammarids, independent of the coexistence with hairychin goby; hairychin goby shift their main food from gammarids to mysids in the presence of red sea bream. However, the degree of this food segregation varies with gammarid supply: more pronounced when gammarid supply is limited, but less pronounced or nonexistent when gammarid supply is abundant. The term "interactive segregation" was defined by Brian (1956) to mean that ecological differences between species are magnified by interaction. In practice, however, it is often difficult to prove that segregation is a direct result of interaction or ecological divergence, as stated by Nilsson (1967). However, the process of food segregation between young red sea bream and hairychin goby in Shijiki Bay shows that interactive segregation occurs between the two species as a result of the dietary shift only by hairychin goby.

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## **Importance of Qualitative Evaluation** of Hatchery-bred Fish for Aquaculture

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### ABSTRACT

Mass production techniques for commercially important species have advanced for a decade in Japan. Numerous hatcheryreared fish are used for not only cage culture but also for releasing programs. However, little information exists on determining the quality and survival potential of hatchery-reared fish. A comparison between reared and wild fish is a prerequisite for the effective use of farmed fingerlings in aquaculture activities. This paper attempts to review, understand, and contrast the biological features of hatchery-reared fish with those of wild fish and to search for the causes of those differences. Various steps in evaluating the quality of reared fish in the hatchery are discussed from a practical viewpoint. In the last decade, artificial propagation of marine animals has been vigorously pursued with a tremendous increase in total production and number of species raised in the hatchery (Fukuhara 1983, Kuronuma and Fukusho 1984, Nose 1985). This phenomena resulted from the diversity of consumer demand which promoted the releasing program to enhance coastal fishery and aquaculture activity.

In Japan, fishermen raised fry from the egg stage for their cage cultures, whereas fish seeds for restocking are produced by the national and prefectural governments. The production of fish for releasing now totals 50 million per year. The major species for planting are red sea bream Pagrus major, Japanese flounder Paralichthys olivaceus, and porgy Acanthopagrus schlegeli (Fig. 1). Despite the great effort in planting artificially reared animals, little information exists on its effectiveness in fisheries after release into the sea. The need for fundamental knowledge of quantity, size, and quality is a major obstacle in considering the effect of releasing activities. More studies on quality evaluation and releasing techniques are urgently needed. The purpose of this paper is to review the qualitative differences between wild and hatchery-reared fish and to emphasize the necessity of qualitative evaluation of hatchery-bred fish in aquaculture.

## Differences between wild and reared fish \_\_\_\_\_

Numerous studies on differences in quality between wild and reared fish have appeared for salmon and trout (e.g., Phillips et al. 1957, Vincent 1960, Wood et al. 1960, Green 1964, Bams 1967, Kobayashi and Ohkuma 1983). Special attention was given to determining measures of quality and to exercising the fry to improve their survival potential in the case of anadromous fish. Concerning marine fish, in spite of their great economic importance, few basic data on biological characteristics of hatchery-raised fish are available. Table 1 shows the differences in biochemical and morphological aspects of sparid fish. Artificially reared fish have been shown in most cases to display a pronounced inferiority to wild fish both in behavior and survival potential. High lipid content is a biochemical feature of reared fish in general, as well as red sea bream. It is uncertain whether the inferiority of reared fish has any effect on survival potential following transfer to the sea.

The general explanation for the poor quality of reared fish is that environmental conditions of reared fish differ largely from those in the wild. In the hatchery, a high percentage of fish can survive due to lack of predation, starvation, and lethal environmental changes, in other words, "selection pressure" (Blaxter 1975). Newly hatched larvae which would die in the wild are capable of feeding on prey and surviving under enhanced rearing conditions. The variation in size can be observed even at the egg stage and also in newly hatched larvae in any species. Morphological differences,



## Figure 1

Species composition of artificial propagation for releasing and cage culture, and total number of fry produced in releasing program. (Source: Annual report of artificial releasing of reared marine seeds, Fishery agency, Jpn. Farm Fish. Assoc.)

Table 1       Biological differences between wild and reared fry in sparid fish					
Species	Items*	Wild	Reared	Source	
Pagrus major	Water Lipid Carbon Hydrogen Nitrogen C/N	78-79% 2-10 mg 35-43% 5.2-6.2% 10.0-12.1% 3.4-3.8	82-72% 3-400 mg 42-49% 5.9-6.7% 9.5-11.6% 3.7-5.0	Anraku and Azeta (1973)	
	Response to threat Abdominal fat content	Sensitive	Weak Higher	Tateishi (1974)	
	Size of fish with complete stripes	11.5 mm SL	9.0 mm SL	Fukuhara and Kuniyuki (1978)	
	Total lipids	1.00 - 1.05	1.95-2.04	Ohshima et al. (1983)	
	Body height, eye diameter, upper jaw length		Smaller	Matsumiya et al. (1984)	
	Muscle freshness		Decreased more rapidly	Iwamoto and Yamanaka (1986	
Oplegnathus fasciatus	Malformation of stripes	19.8%	23.2-81.3%	Fukusho (1979)	

particularly size at early life stage, lead to size hierarchy and differences in fish activities (Yamagishi 1964).

Morphological changes in eggs and larvae depend largely on the nutritional conditions of spawning fish of red sea bream (Watanabe et al. 1984). To observe the survival curve, Keitoku et al. (1985) conducted a starvation experiment of larval red sea bream maintained simultaneously in a largescale tank used for artificial propagation. The starvation experiment indicated the existence of poor-quality larvae which died prior to the point of no return (PNR) (Fig. 2), and a close relationship was found between survival in the starvation test and percent survival of fish at harvest in the hatchery production unit. These findings suggested the source of difference in fish quality is related to the health of broodstock or developmental stages of newly hatched larvae. In addition, larvae are free from starvation and predation pressure in the rearing tank, which allows non-exercised fish to avoid various lethal conditions and experience a high survival rate. Biological characteristics of non-exercised fish become fixed as the rearing period lengthens, leading to a loss of wildness and to an inability of the hatchery-reared fish to adjust to new surroundings after transfer (Azuma 1974, Blaxter 1975).

## Determining fish quality \_\_\_\_\_

Stamina tunnels or swimming performance have been used to evaluate the quality of planted salmon and trout (e.g., Vincent 1960, Green 1964, Bams 1967). As for marine fish, various examinations have been used to determine fish quality: rheotaxis, resistance to exposure, starvation, low oxygen concentrations, and narcotization (Kitajima et al. 1980, Ohgami and Suzuki 1983, Keitoku et al. 1985, Maruyama 1985). An exposure test was employed to compare the activity of reared juveniles in a nutritional experiment. Mortality and recovery time of examined juveniles usually correlate with nutritional conditions (Kitajima et al. 1978, 1980). Maruyama (1985) compared mortality and recovery time, following a 2-minute exposure, between conventionally reared fish and semi-wild fish reared in an earthen pond. One minute after exposure, 80% of the semi-wild fish recovered with no mortality. On the other hand, less than 25% of the hatchery-reared fish recovered after 1 minute with 50% mortality. These observations suggest that an exposure test is available which may predict the activity of reared fish under different conditions, and that the differences are caused by different habitat and/or food items previously ingested.

In Ayu fish *Plecoglossus altivelis*, a freshwater species, swimming performance is usually employed to assess the migrant behavior of hatchery-reared fish (Fig. 3). Fish raised under lotic conditions showed a higher percentage of migrant behavior than those under lentic conditions. Adjustment to water current of 30–60 cm/sec prior to planting is effective for this species in increasing upstream movement (Hiroshima Prefect. Freshwater Fish Cent. 1983).





#### **Conclusions**.

It can be reasoned that qualitative differences exist between reared and wild fish if biotic and abiotic conditions are compared. The causes of differences in both animals are mainly attributable to differences in feeding and habitat situations. Hatchery-reared fish are immediately subjected to reduced food levels and the threat of predation following transplantation. To alleviate various stresses to newly introduced fish, some manipulation (such as exposure to moving water, predators, and starvation) is required during the course of rearing or before planting. Fish exposed to predators were less vulnerable to predation, resulting in decreased fry mortality (Ginetz and Larkin 1976). Henderson (1980) emphasized the necessity of certain durations for transplanted fish: periods of recovery of normal movement, familiarization with the new habitat, and adjustment of feeding habit. As already mentioned for Ayu fish, exercise in a water current is a profitable strategy prior to planting. Swimming exercise increased endurance in trained coho salmon compared with control groups, and the effect of exercise was maintained for 2 months (Besner and Smith 1983).

These studies indicate that some manipulations are effective in exercising and ajusting reared fry prior to release in a new habitat. As for marine fish production, to improve the quality of reared fish and to increase the survival potential after planting, the inferiority of laboratory-reared fish must be prevented during rearing (Fig. 4). The concept of



Figure 3 Diagram of apparatus to determine migrant behavior in Ayu fish.



Figure 4 Concept for evaluating and improving fish quality in artificial propagation and releasing procedures.

exercise and adjustment is not currently employed in rearing procedures from egg stage to young. Starvation tests of newly hatched larvae before reaching PNR indicate the possibility of selecting viable larvae for production purposes (Fukuhara 1974, Keitoku et al. 1985). Experimentally, hatchery fish reared in a water current are usually resistant to handling procedures during transfer.

In Japan, intermediate rearing of marine fish is generally conducted in net cages before their release. This intermediate rearing is aimed in most cases at improving the effectiveness of the rearing facility and increasing fish length prior to release. Exercise and exposure to starvation and predation are needed to improve fish quality in the intermediate rearing strategy. Quality determinations of reared fish become more important to the evaluation of artificial recruitment effects as releasing activities are carried out vigorously for various marine fishes in the future. In turn, releasing techniques must be established to produce planting seeds which meed the biological characteristics needed for aquaculture ventures.

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## Recent Progress in Artificial Propagation of Marine Species for Japanese Sea-farming and Aquaculture

#### **AKIRA SUDA**

Japan Sea Farming Association Kanda-Ogawamachi, 2-12, Chiyoda-Ku Tokyo, Japan In the last decade, with changes in social and economic conditions, including the imposition of 200-mile territorial waters by many countries, the roles of sea-farming and aquaculture have become more important in the coastal fisheries of Japan. In this connection, considerable efforts have been directed by governments and individuals to develop techniques of artificial propagation, which are the basis of fish farming and aquaculture. As a result, considerable progress has been made in this field.

As illustrated in Figure 1, in the 7 years during 1977-84, larval rearing of various species has steadily increased in number (unpublished Prefectural and Fishery Agency reports). In 1984 production of juveniles was attained in 32 species of fishes, 15 species of crustaceans, 21 species of shellfishes, and 9 miscellaneous species. Production has also increased in total numbers as well (see Figure 2): 3.8 times for red sea bream *Pagrus major*, more than 40 times for Japanese flounder *Paralichthys olivaceus*, about 5 times for blue crab *Portunus trituberculatus*, and 3.8 times for abalones (*Haliotis* spp.). The production of Kuruma prawns *Penaeus japonicus* and Yesso scallop *Patinopecten yessoensis* had already attained high levels by the mid-1970s and increased another 20% by 1984.

Production of juveniles in 1984, the latest year for which data are available, is shown in Table 1 by type of utilization and hatchery facility (Fishery Agency and Japan Sea Farming Assoc. 1986). Research on larval rearing is carried out by the following facilities which play different roles in production:

1 National (A) and local (B) governmental facilites mainly address the basic, practical aspects of technical development.

2 Local governmental (B) hatcheries disseminate results of technical developments to fishermen.



Figure 1 Yearly change in number of species in seed production, 1977–84.



Figure 2 Yearly change in number of seeds produced, 1977–84.

**3** Fishermens' association (C) and private (D) hatcheries produce seed for industrial operations.

A great number of scallop seeds are produced exclusively by group-C hatcheries on a commercial basis for restocking operations and aquaculture. Fingerlings of red sea bream and Japanese flounder and Kuruma prawn seed are produced in many hatcheries of all classifications and are used in practical and experimental restocking operations as well as in industrial aquaculture. In aquaculture of these species, artificially produced seeds are now contributing a larger share of seed stock. Blue crabs are reared mainly by government (groups A and B) hatcheries, thus seed is not directed to aquaculture but to restocking operations of either a more practical or experimental nature. In the case of the Tiger puffer *Fugu rubripes*, fingerlings are produced almost solely for aquaculture. Various technical problems are still unsolved for other species, and government (A and B) hatcheries are

Table 1       Number (10 <sup>3</sup> ) of seeds produced in 1984.							
Species	Type of utilization		Type of facilities				
	Sea-farming	Aquaculture	A	В	С	D	
Herring	570		416	154			
Striped jack	39	197	39	1		196	
Yellowtail	1,210		1,210				
Jack mackerel	257		257				
Striped knifejaw	880	542		1,338	54	30	
Japanese seabass	521	30		551			
Three-line grunt	811		219	592			
Red-spotted grouper	49		49				
Black sea bream	6,307	1,225		6,562	177	79:	
Red sea bream	22,572	16,742	4,245	23,908	2,446	8,71	
Black rockfish	717	150	717	145	5		
Scorpionfish	240			240			
Japanese flounder	8,483	5,264	2,567	6,845	87	4,18	
Common flounder	2,972			2,972			
Tiger puffer	757	1,622		2,073		30	
Kuruma prawn	484,684	117,165	90,000	352,146	11,745	147,95	
Kuma prawn	3,345	1992 (11 199 <b>8</b> ) (2008) (2019)		1,345	2,000		
Yoshi prawn	35,927			33,093	Constant of Decision (Co	2,83	
Hanasaki kingcrab	426		426				
Mad crab	1,053		1,053				
Blue crab	38,350		13,350	23,944	316	74	
Abalones	29,960	1,002		26,572	1,813	2,39	
Horned turban	367	5		372	-,	-,-,	
Japanese babylon	749	_		745	4		
Ark shell	5,370	8,480		13,340	500	1	
Pearl oyster	- ,	23,568		12,740	5,028	5,80	
Noble scallop		2,104		1,714	-,0	39	
Baking scallop	426	893		426	893		
Yesso scallop	1,777,710	1,490,093		.20	3,267,803		
Clam shell	1,241	-,,		1,241			
Sea urchins	4,279	70		4,025	324		
Sea urennis	4,217	70		4,025	524		

Group B: Local governmental hatcheries

Group C: Fishermen's association hatcheries

Group D: Private hatcheries

Table 2       Efficiencies of rearing techniques represented by survival rate and number of seeds per cubic meter at the end of production.					
Species	Size of seed (mm)	Survival rate (%)	Number of seed per m <sup>3</sup>		
Yellowtail	20	10	500		
Red sea bream	20	35-40	5,000		
Three-line grunt	17	60	2,500		
Black rockfish	30	60	1,690		
Japanese flounder	20	60	5,000		
Herring	50	60	3,000		
Kuruma prawn	13	70	18,000		
Blue crab	Crab stage 1	25-40	6,000-10,000		
Hanasaki kingcrab	Crab stage 1	60	7,500		

involved in basic studies. For such groups, the development of rearing techniques for species not previously cultured is a major responsibility.

# Recent views of rearing techniques \_\_\_\_\_

## Efficiency

Nine selected species from the Japan Sea Farming Association are presented in Table 2 to show the efficiency of rearing techniques, represented by (1) survival rates throughout the process of larval rearing and (2) number of larvae reared per cubic meter at the end of the process (Japan Sea Farming Assoc. 1984). Survival rates are more than 30%, exceeding 50% in many species. More efficient rearing of some species has been reported from some of the hatcheries in group B. Intensive mass production of juveniles with high survival rates is becoming practical for red sea bream, Japanese flounder, Kuruma prawn, and blue crab in many Japanese hatcheries, although some problems still remain.

## Areas needing improvement

One basic problem is that the survival rate of larvae fluctuates widely in every rearing operation even within the same facility. Moreover, as is shown in Figure 3, survival rates vary among years and hatcheries (Japan Sea Farming Assoc. 1983, 1984, 1985; unpubl. Prefectural and Fishery Agency reports). Such fluctuations are due to many uncontrolled causes such as quality of brood fish, conditions in the rearing tank, food quality, occurrence of disease, and cannibalism.

Remarkable difficulties occur with some species for which mass production of fingerlings is becoming practical. For example, body deformity with abnormal pigmentation frequently occurs in flounder and flatfishes. Also, severe losses of larvae occur during the rearing of Kuruma prawn caused by baculovirus disease. Usually special problems occur in



Figure 3 Fluctuation of survival rate (%) at the end of seed production among hatcheries and years, 1982–84.

raising particular species, including malnutrition of rotifers and cannibalism of yellowtail. For these problems, answers have yet to be found.

Another basic problem is that many more fingerlings are needed to develop restocking operations, because the number produced by natural recruitment is greater than that of fingerlings released. Also, we have no conclusive evidence of the ability of fingerlings to survive in the wild. This ability is essential for the success of restocking operations. The matter will be observed further in the discussion on restocking of red sea bream.

# An example of a restocking operation \_\_\_\_\_

Currently, restocking operations on some commercial species, e.g., Kuruma prawn, blue crab, scallops, red sea bream, and flounder, are being undertaken in various areas in Japan. Among them, the operation on Yesso scallop resulted in a remarkable catch increase from 15 thousand tons in the



Figure 4 Number (10<sup>3</sup>) of fingerlings of red sea bream released, by prefecture, 1984.

mid-1940s to more than 120 thousand tons in 1984 (Bureau of Statistics 1985). As to other species, generally speaking, the effects of stocking have not yet proven statistically significant. Still, some local stocks, when combined with favorable natural conditions and the efforts of people involved, have been effectively restocked.

Following is an outline of the red sea bream restocking operations, as an example for discussion.

1 Before release, fingerlings are kept for a short time in a pen at the location where they will be released to acclimatize them to the wild environment. Figure 4 illustrates the number of fingerlings released by each prefecture (Fishery Agency and Japan Sea Farming Assoc. 1986). To decide the scale of release, it is important to know whether the amount of release is large enough compared with that of natural recruitment. To arrange the key to this question, the ratio of the number of fingerlings released to the number caught locally is given in Figure 5. When the ratio is less than 1, the amount of release is expected to be less than natural recruitment.



Figure 5 Ratio of number of fingerlings released to that of catch in number, by prefecture, 1984.

$$C = R \cdot F/Z$$
  $R'/C = R'/(R \cdot F/Z)$ 

If R'/C < 1, then R'/R < F/Z < 1

where R = number of natural recruitment,

- R' = number of fingerlings released,
- C = number of catch in a given year, including all age groups,
- F = instantaneous rate of fishing mortality, and
- Z = instantaneous rate of total mortality.

In many cases, the ratio is less than 1 which suggests more fingerlings are necessary before the beneficial effects of the stocking operation are obvious (Fig. 5) (Bureau of Statistics 1985, Fishery Agency and Japan Sea Farming Assoc. 1986).

2 In Areas 1 and 2 in Figure 5, the ratio is fairly high, especially in Area 1 where the catch actually increased recently by about 20% (~10 tons in weight) (Kanagawa Prefect. Fish. Exp. Stn. 1986). Here, the catch by sportfishing is

estimated at 30–35 tons. If the latter estimate is taken into consideration, the increase in catch should be much more. In Area 2, surveys of fish markets indicated that about 10% of the catch came from fingerlings released (Hiroshima Prefect. Fish Exp. Stn. 1985).

**3** The quality of fingerlings must be examined. It is expected that the difference in inherent abilities of fingerlings to survive the wild environment affects the efficiency of the operation. Recently, attempts have been made to grow larvae of red sea bream in an extensive rearing system in a large outdoor pond without an artificial diet. Some endurance tests on larvae grown in this way suggest that fingerlings reared in extensive culture survive in the wild better than those reared in intensive culture. This also suggests we can enhance the survival ability through the manner of rearing (Japan Sea Farming Assoc. 1985).

4 Cost of fingerlings is also an important factor for a practical operation. In 1984, the cost for group-B hatcheries ranged between 3 and 50 yen, with a mean value of 13.5 yen, per fish. Preliminary calculations of total expenditures to restock a fingerling, though still uncertain, suggest that it costs two to three times and more than the expenditures of rearng alone. The cost of fingerling production, together with other restocking costs, must be reduced if the operation is to be more cost-effective. Thus, more improvements in rearing techniques are needed for the healthy development of a restocking program.

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