ECOLOGY OF AQUACULTURE SPECIES AND ENHANCEMENT OF STOCKS

PROCEEDINGS OF THE THIRTIETH U.S. - JAPAN MEETING ON AQUACULTURE

Sarasota, FL U.S.A
December 3 and 4, 2001

Edited by

Yasuaki Nakamura, James P. McVey,
Ken Leber, Carole Neidig,
Sondra Fox and Kim Churchill

Mote Marine Laboratory
Center for Fisheries Enhancement

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

UJNR Aquaculture Panel Chairmen:
Yasuaki Nakamura, Japan
James P. McVey, United States

Correct citation of this Report is:

Nakamura, Y., J.P. McVey, K. Leber, C. Neidig, S. Fox, and K. Churchill, (eds.). 2003. Ecology of Aquaculture Species and Enhancement of Stocks. Proceedings of the Thirtieth U.S. – Japan Meeting on Aquaculture. Sarasota, Florida, 3-4 December. UJNR Technical Report No. 30. Sarasota, FL: Mote Marine Laboratory.

An electronic version of this Report is available online at:
NOAA Library UJNR Aquaculture Panel
http://www.lib.noaa.gov/japan/aquaculture/aquaculture_panel.htm

Print copies of this Report can be obtained from:

Mote Marine Laboratory	Florida Sea Grant
Center For Fisheries Enhancement	University of Florida
1600 Ken Thompson Pkwy	PO Box 110409
Sarasota, FL 34236	Gainesville, FL 110409
(941) 388-4441	(352) 392-2801
(941) 388-6461 (fax)	(352) 392-5113 (fax)

ECOLOGY OF AQUACULTURE SPECIES AND ENHANCEMENT OF STOCKS

PROCEEDINGS OF THE THIRTIETH U.S. – JAPAN MEETING ON AQUACULTURE

EDITED BY
YASUAKI NAKAMURA, JAMES P. MCVEY,
KEN LEBER, CAROLE NEIDIG,
SONDRA FOX, AND KIM CHURCHILL

■ Contents

◼ PREFACE

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

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

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

The 30th U.S.-Japan Aquaculture Panel Business Meeting, a scientific symposium, and several field trips were held from December 2-7 2001 at the Mote Marine Laboratory in Sarasota, Florida and at sites throughout Central Florida. The scientific symposium was organized by Dr. Kenneth Leber and his staff at Mote Marine Laboratory. The editors gratefully acknowledge the many hours of hard work by Sondra Fox and Kim Churchill spent editing and compiling these proceedings.

Yasuaki Nakamura – Japan
James P. McVey – United States

I. U.S.-Japan Cooperation

Open Ocean Aquaculture – a Venue for Cooperative Research Between the United States and Japan

Charles E. Helsley
Sea Grant College Program
University of Hawaii at Manoa
2525 Correa Road
Honolulu, Hawaii 96822
USA

During the past decade, aquaculture in the United States has begun to assume a more significant role both in U.S. policy and in the U.S. economy. In 1999 the U.S. Department of Commerce (DOC) enunciated a policy to encourage aquaculture development in the United States. This plan envisages the growth of the aquaculture industry from the current value of $900 million to $5 billion by 2025. It will involve a five-fold production increase in order to reduce the seafood import deficit and stabilize world seafood supplies. Success of the plan will depend upon rapid removal of administrative and scientific impediments.

At the time the plan was promulgated, aquaculture in the U.S. was primarily a land-based activity utilizing fresh water ponds and tanks constructed along or near rivers. Catfish production was ten-fold that of other species and grew more than 40% during the past decade. However, during the same decade several other species declined, especially those grown in estuaries, such as the American Oyster, or in flood plains along rivers, such as crawfish. These declines probably resulted from price competition, decreasing water quality, habitat destruction, and land use changes.

The expansion of marine organism production on near-shore land will likely be limited, as it is already in high demand and expensive. Freshwater expansion is also likely to decrease as readily accessible areas are already being utilized. Near-shore protected waters are generally areas of substantial use conflict. They are currently already at or near carrying capacity due to the abundant sources of nutrients that can be drawn from nearby upland areas.

In the past five years, there have been several attempts to make greater use of our near-shore marine waters, particularly for salmon, clams, oysters and shrimp. As a result of changes in national policy, marine aquaculture, both near-shore and offshore, is poised for significant expansion during the next decade. But in near-shore waters, this expansion faces significant opposition primarily by those that believe these waters are common areas and already used to capacity.

Background

The geography of the coastal U.S. can be subdivided into four general classes: sandy beaches, coastal estuaries, glaciated areas with fjords, and rugged rocky coasts generally with few embayments. Each of these areas is heavily urbanized since half of the U.S. population lives within 200 km of the coast.

Urbanization brings with it the problems of runoff from paved areas, discharge of industrial and human wastes and a general diminution of water quality. Combining urban

discharge with agricultural runoff substantially increases the possibility of nutrient enrichment of coastal waters. This problem now exists in several of the largest estuarine systems in the United States. The aquaculture of species other than filter feeders (clams, oysters, menhaden) or detritivores (mullet, shrimp) is unlikely to be encouraged in these areas.

Sandy beach coasts are generally areas of high wave activity, especially during storms. Moreover, the water tends to be relatively shallow for long distances offshore and in many areas in the Gulf of Mexico and the Atlantic seaboard one must go 50 to 100 k from shore before waters of 20 m depth is reached. Thus, aquaculture in these areas is restricted to the growing of various benthic organisms.

Glaciated areas have many fjords developed in deeply embayed coastlines. This type of coastline is present in New England from New York City to the Canadian border, the Puget Sound area of Washington State, and in Alaska. Most of the marine aquaculture effort has been placed in these areas and appear at first glance to be ideal for near-shore, sheltered water development. However, there are many conflicting demands on these waters, most notably the 'not in my front yard' syndrome exercised by wealthy landowners who value the pristine views of the bay. Several political jurisdictions, especially in Alaska, greatly restrict or prohibit all forms of net pen aquaculture.

The rocky and mountainous coasts of the western U.S. are generally highly exposed with few sheltered areas and even fewer port facilities. These appear to be unlikely targets for near-shore marine aquaculture development in the near future.

But there are sites in the offshore in all regions that may be suitable areas for aquaculture. Of particular interest are those locations where the water depth is deep enough to assure circulation but shallow enough to enable anchoring to take place.

From a geographical perspective it is unlikely that finfish aquaculture will expand very much in near-shore waters for these are areas where conflict is greatest and public concern for water quality is likely to become an insurmountable obstacle. Substantial expansion may be possible in areas further offshore, but even here, it will require acceptance by consumers and the rapid removal of administrative impediments currently present in the permitting process. It also will require the development and implementation of sustainable new production technologies.

The Challenges

Although offshore areas appear attractive, many challenges must be overcome to make use of these areas. These challenges fall into at least four classes: (1) Social – public acceptance, jurisdiction, and permitting; (2) Technological – development of cages, culture and feeding systems, harvesting systems, etc., (3) Biological – development of new species, use of indigenous species, adequacy of food supply, management of disease and (4) Managerial – issuance and monitoring of leases, requirements for routine monitoring of effluents, and the development of the best management approach to husbandry practices. In addition, one must consider a series of infrastructure issues (ports, hatchery, and processing facilities) and marketing issues. Clearly, there are enough challenges to go around.

From the social perspective, it is likely that environmental critics will challenge the development of offshore areas on several grounds. Questions that might be asked are: What happens to the waste products? Will water quality be impacted? Are blooms of phytoplankton likely? Will they engender harmful algal blooms? Will the accidental release of fish from offshore fish farms endanger native stocks? What are we going to feed the fish? Will we

endanger other areas in doing so? Will the farms be harbingers of disease? What technologies are to be used to ameliorate these concerns?

One would think that experiences from elsewhere in the world could offer solutions to these public concerns. But unfortunately most current literature focuses on the failures rather than the successes of these programs. Many more case histories of success are needed with adequate documentation of the environmental impacts, so that factual material is available to counter the critics. Needed most importantly is evidence of benign or beneficial interactions with the environment rather than reports that focus solely on the devastation of accidental releases, eutrophication due to over feeding or overproduction in a confined body of water.

The technology of growing fish in cages should be shifted from a systems point of view to an emphasis on strengthening the weakest links in the system. Until recently, offshore aquaculture was impractical because the cages would not stand the stress of the high-energy offshore environment. With better design and use of stronger and better-engineered materials this constraint has been largely removed. The questions now become: Can the fish stand this high-energy environment? Can the offshore environment accept this added load on its carrying capacity? Can we develop efficient means of feeding and harvesting fish in these higher energy environments? Can we make use of a systems approach for integrating species, cages, hatcheries, harvesting, and waste recycling? What are the economics involved in a move to far-offshore environments?

Understanding the reproductive biology of indigenous species is an essential component of aquaculture development in much of the U.S. since the use of non-indigenous stocks is likely to meet with considerable opposition. Moreover, the biological interaction between the cultured stock and the wild stock needs to be understood. We need much more understanding of the use of polyculture in waste management and disease control as well.

Management strategies in the U.S. are virtually non-existent and all decisions are made on a case-by-case basis. We need to develop a more uniform management approach in which an agency takes the lead role rather than all agencies asserting their right to control portions of the development. Aquaculture must be understood as an agricultural activity in which certain changes are permitted and expected. Moreover, we need to carefully assess the carrying capacity of offshore waters and to understand the role of waste products in this environment where circulation is high and concentration of nutrients and particulates is low. Clearly, "too much" is "too much" in any environment, but we currently have no idea how to define "too much" in any qualitative or quantitative way.

Research Needs

To assist in meeting the long-term Department of Commerce objectives, a number of activities need to be taken on a coordinated basis. Among the many possibilities that could be addressed to expedite the growth of an offshore aquaculture activity, more than a dozen major areas of research immediately come to mind. These include:

- Permitting – The permitting process is cumbersome and considerable agency overlap exists. This makes it "expensive" to get started. The process needs to be clarified and streamlined, perhaps by simply making it a parallel process rather than a serial one. Redundancy and overlap in jurisdiction between agencies also needs to be minimized.

- Environmental Impact – The interaction of an open ocean cage culture farm with multiple cages has not been studied adequately and considerable uncertainty exists as to the short term and long term environmental effects of one or more cages or farms in the oligotrophic waters of the tropics. As the first few farms develop, they must be carefully observed to determine their interaction with the ecosystem external to the cage including nearby coral reefs. There is no reason to expect adverse interactions at the levels of discharge expected but this needs to be verified.

- Environmental Monitoring – Current methods of monitoring of nutrients in oligotophic waters are both time consuming and expensive. Methods should be found to monitor appropriate compounds, organisms, or proxies on an instrumental probe or remote-sensing basis rather than to collect individual water samples for future laboratory analysis.

- Nutrition and Animal Husbandry – Only a few tropical species have life cycles that are well enough understood to permit reliable culture. Even though we can culture many of these to marketable size, we do not understand the nutritional needs or animal behavior and husbandry issues well enough to reliably culture them in an economic fashion.

- Hatchery Development – Offshore farms must be able to produce large numbers of fingerlings of uniform size. Currently few hatcheries are capable of this. Many are needed in order to minimize risk to all farmers. Increased understanding of hatchery technology and methodology is also highly desirable. The effect of culture density on juvenile size also needs to be more fully understood.

- Diversity of Brood-Stocks – Multiple sites need to begin to maintain brood-stock of all cultured species in any given area. These stocks need to be maintained with a genetic makeup that is equivalent to that of the wild stock (just in case a cage breaks up and releases a large number of fish).

- Hatchery Technologies- Hatchery technologies are often developed at a laboratory scale. But the methods developed in the laboratory need to be field tested to determine their efficiency and/or profitability under "real world" conditions.

- Cultured Species – The number of species under culture must be increased. A diversity of species is needed to avoid flooding the market with any one species. Moreover, some species may grow faster than others and thus appear ideal, but the market demands variety and thus many species are likely to be needed to sustain a viable open ocean aquaculture operation.

- Life history – Life history investigations on many invertebrates and vertebrates used in the marine ornamental trade need to be studied in a systematic approach to develop culture technologies and/or management strategies for sustainable harvest.

- Market Development – Marketing is one of the most critical components in the economic success of offshore aquaculture project. Reliable demand driven markets need to be developed for each species under culture.

- Disease Diagnosis and Prevention – Disease diagnosis and prevention is generally done in hindsight. In most aquaculture operations, it is only addressed once disease has become a major problem. In most cultured seafood activities disease appears as the operation expands to higher densities in the same limited area. Although this is most recognizable in shrimp, it is also present in salmon and can be expected to become an issue for all cultured species. We need to understand disease pathogens before, not after, they become the "make or break" economic topic.

- Feed and Harvesting Technology – Feed and harvesting technology in large part has been developed for surface cages in sheltered water environments. Open ocean culture of fish will require changes in these established practices for the operation will of necessity have to be undertaken in more remote locations and will regularly involve working in adverse and even hazardous conditions. Many operations will require long hours at sea and will often require divers on a regular or routine basis. Automation of feed delivery systems and harvest systems is needed to minimize these costs and hazards.

- Policy and Regulation Issues – Policy and regulations governing the discharge from confined farm animal facilities in the U.S. were developed from a land culture perspective. These policies and rules have been transferred to the ocean realm without considering the change in the environment or natural living conditions of the species under culture. The rules for confined culture of farmed animals on land does not readily adapt itself to offshore conditions where prodigious quantities of water flow through the facility and the water itself is the medium in which the organism is being grown. The standard policies and regulations need to be changed to accommodate this new industry.

- Education – Public education is essential to the development of a friendly consumer and accepting public. Currently, educational materials for marine aquaculture are lacking and there is need for both the development of educational material for the general public and for those that intend to make this topic their profession. Support for specialists to assist in the resolution of problems that entrepreneurs in this field may encounter is also needed.

Current Sea Grant Sponsored Research Activities

As part of the National Marine Aquaculture Initiative program, Sea Grant programs throughout the U.S. are initiating new research programs focused on marine and offshore aquaculture. Many new species are currently being studied. These include: halibut, haddock, and cod at the University of New Hampshire; black sea bass at the University of South Carolina; mutton snapper at University of Miami and the University of North Carolina; cobia at the Universities of Virginia, South Carolina, Mississippi, and Texas; yellow tail snapper at the University of Texas; sable fish (a cod) and cardinal rockfish at the NMFS Mansfield Laboratory; corvina at the University of California; and bay scallops at the NMFS Milford Laboratory and the University of South Florida.

At many institutions additional marine species are under culture using other sources of funding. In Hawaii these include amberjack, opakapaka (a deep-water snapper), Gulf of Mexico red snapper, moi (pacific threadfin), mahimahi, striped mullet, milkfish, kumu (a goatfish) and a number of marine ornamentals. There are environmental studies on an active offshore farm at the University of Hawaii, animal husbandry and nutrition studies at the Oceanic Institute, and studies of cage design and anchor systems at the University of New Hampshire and MIT. MIT is also developing designs for automated feeders and the culture of flounder in closed circulation systems at the University of Connecticut and at North Carolina State University.

The Role of the UJNR Aquaculture Panel

Given the expected developments in research and commercial farms throughout the U.S. over the next few years, it is imperative to address the issues summarized above as soon as possible. Many of these are topics have been the subject of cooperative research and information exchange within UJNR in the past and remain issues for future cooperative efforts. Hatchery technology advanced culture technologies, culture of new species, identification and control of disease organisms are obvious examples. New areas of study might include development of cages and husbandry technology for rough water environments, identification of alternative food sources, understanding of nutrition requirements, definition of carrying capacity of coastal and offshore waters, development and application of environmental monitoring technology, consideration of regulatory and policy issues, and ocean ranching. Each of these topics would benefit from multidisciplinary approaches to science and the exchange of ideas from diverse experience bases. The broad topics mentioned would provide opportunities for scientific exchange particularly between young scientists with ideas and technologies at the borders of the "tried and true" conventional methodologies. All of these areas appear eminently suitable for future UJNR efforts

Cooperation between researchers in the U.S. and Japan is highly desirable to accomplish these goals. The cooperative international scientific information exchange program fostered by the UJNR Aquaculture program has enabled many U.S. scientists to become more aware of Japan's extensive experience and research base in marine aquaculture. The development of an active marine aquaculture research program in the U.S. presents an opportunity to expand upon those ties and to provide information useful to both countries as we attempt to make more utilization of the sea. Possible areas of further scientific research and information exchange include: (1) establishment of culture protocol for additional species, such as cold water, deep water, tropical and subtropical species, (2) establishing guidelines for the assessment of carrying capacity of coastal and offshore waters; (3) identification of additional sources of high protein fish food and development of additional additives and nutrition enhancers and (4) the development of technologies that would assist in the growth of the aquaculture industry in more exposed locations.

Conclusion

The research and exchange of ideas related to the extension of current aquacultural activities into more exposed and high-energy environments, generally referred to as Open Ocean Aquaculture, appears to be a fruitful area for future UJNR activities. The breadth of topics is appropriate, the need is present, and the growing commitments within the U.S. all suggest that this would be an appropriate theme for future exchanges.

■ II. GROWTH, NUTRITION AND GENETIC DIVERSITY

Daily Ration of Hatchery-Reared Japanese Flounder *Paralichthys olivaceus* as an Indicator of Release Place, Time and Fry Quality. *In situ* Direct Estimation and Possibility of New Methods by Stable Isotope

Osamu Tominaga
Tadahisa Seikai
Fukui Prefectural University
1-1, Gakuen-cho, Obama,
Fukui 917-0003
JAPAN

Kinya Nogami
Japan Sea-Farming Association
3-14-8, Uchikanda, Chiyoda
Tokyo 101-0047
JAPAN

Tatsuo Tsusaki
Yasushi Hondo
Naoto Murakami
Japan Sea-Farming Association Miyazu Station
1721 Odashukuno, Miyazu
Kyoto 626-0052
JAPAN

Yousuke Tanaka
Masaru Tanaka
Kyoto University
Oiwake-cho, Kitashirakawa, Kyoto
Kyoto 606-8502
JAPAN

Abstract

In order to examine the availability of daily ration as an indicator of release place, time and fry quality, mass releases of hatchery-reared Japanese flounder *Paralichthys olivaceus* were conducted under various experiment conditions in Wakasa Bay, the Sea of Japan in 1997 and 1998. Daily ration was estimated in terms of percent of body weight by Elliott and Persson's method in the field. Daily ration was between 1.5% BW/day and 22.2% BW/day and closely related to the food availability, especially mysids biomass. Daily ration of wild flounder was 3 times as much as that of released fish in July 1997. Judging from these results, daily ration is thought to reflect food availability in the release area and seedling quality, and be used as an indicator to evaluate the quality of the release technique.

Elliott and Persson's method does not give the daily ration of individual fish, and provides food consumption during only one limited day. It is more important to know the accumulated food consumption after release and the ration on an individual basis. We tried to estimate the accumulated food consumption from the temporal change in carbon stable isotope ratio (δ^{13}C) in dorsal muscle when the diet of juveniles was switched from the formula feed to live mysids (different δ^{13}C from the formula feed). The average δ^{13}C of experiment group fed mysids rapidly increased from -20.70‰ to -19.19‰ during the experiment (14 days). Our results suggested that the stable isotope could be a useful tool to estimate feeding condition after release.

Japanese flounder *Paralichthys olivaceus* is one of the most important target species of stock enhancement in Japan. The release size of hatchery-reared flounder is an important factor affecting survival after release, (Fujita *et al.,* 1993; Yamashita *et al.*, 1994; Tominaga and Watanabe, 1998). In order to improve the stocking effect of hatchery-reared juveniles, it is

necessary to determine the optimum release place and time. Food availability for stocked juveniles is a key factor to evaluate the quality of release tactics. However, it is not easy to estimate the biomass of food organisms in the release area, because of the difficulty in quantitative sampling. Feeding intensity of released juveniles is affected by not only healthy condition of fish (vitality and nutritional status), but also physical, biological, and environmental conditions in the release area. Therefore, daily ration of fish after release becomes the useful tool to evaluate the release place, time and fry quality.

The quantity of food consumed by fish is commonly estimated on a daily basis. The daily ration model of Elliott and Persson's method (Elliott and Persson, 1978) is widely accepted as the most theoretically rigorous (Cochran, 1979; Eggers, 1979; Elliott, 1979). This method does not require a special instrument and is easy to apply for field investigations. However, it does not give the daily ration of individual fish, and provides food consumption during only one limited day. It is more important to know the accumulated food consumption after release and the ration on an individual basis.

Stable carbon and nitrogen isotope analysis is being used in the ecological study of energy flow because stable isotopic compositions of consumer tissues can often be related predictably to stable isotopic compositions of diet (DeNiro and Epstein, 1978). Hesslein *et al.* (1993) investigated the response of the isotopic compositions of broad whitefish *Coregonus nasus* to change in the isotopic compositions in their diet. They concluded that in the rapid growth fish, the changes of the stable isotope ratio of sulfur, carbon and nitrogen may be high and the rate of change would directly reflect growth ratio.

Japanese flounder juveniles grow fast and the diet of released fish drastically changes from artificial pellets to live organisms, mainly mysids and larval and juvenile fish. We speculated that the stable isotope ratio of carbon in the tissue of the released juveniles was approaching those of prey organisms in the release area and the more the released fish consumed prey, the faster the ratio of stable isotope change. We have no data available to determine how long it would take for the dorsal muscle to respond isotopically to the new food source.

In the present study, mass releases of hatchery-reared Japanese flounder were conducted under various experimental conditions in Wakasa Bay, the Sea of Japan, from 1997 to 1998. Daily ration of a week after release was estimated in terms of percent of body weight (BW) by Elliott and Persson's method in the field. We investigated the preliminary experiment for temporal change in the stable isotope ratio of $\delta^{13}C$ in the dorsal muscle when the diet of juveniles was switched from the artificial pellet to live mysids.

Materials and Methods

Field Experiment

Mass releases were conducted at 1-2 m depth in Wada beach, Wakasa Bay mid coastal area of the Sea of Japan in 1997 and 1998 (Fig. 1, Table 1). In 1997, forty thousand flounder juveniles were released on 29 May (early group) and 2 July (late group), respectively (80,000 in total). Average total length (TL) of the early and late group was 53.5 mm and 51.8 mm, respectively.
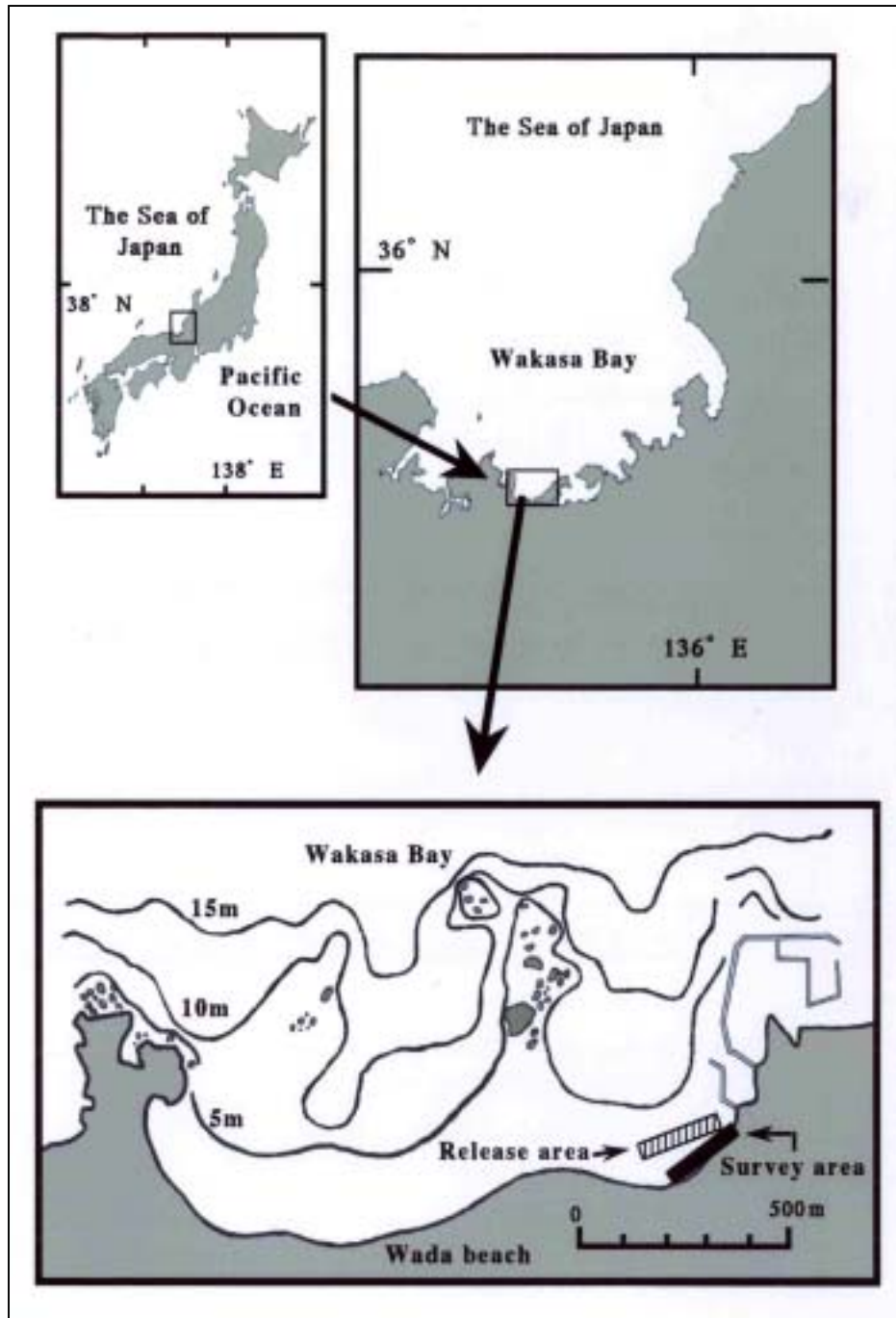
Figure 1. Map of Wada beach, Wakasa Bay, the Sea of Japan, showing locations of flounder release and 24-hour surveys.

Table 1. Date, size, number of individual, environmental conditions of releases for hatchery-reared Japanese flounder Paralicthys olivaceus juveniles conducted in 1997 and 1998

	1997		1998	
	Early group	Late group	Large Group	Small group
Date of release	29.May	2.Jul	21.May	
Release size	53.5mmTL	51.8mmTL	60.7mmTL	37.1mmTL
No. of fish released	40,000	40,000	50,000	50,000
Release area				
Water depth	1.5m	2m	2m	
Water temperature	19℃	24℃	21℃	

In 1998, two different size groups of flounder of which the mean total length was 60.7 mm TL (large group) and 37.1 mm TL (small group) were stocked on 21 May. The number of individuals released was 50,000 each. Hatchery-reared fish were marked on the otoliths by alizarin complexone (ALC). The large and small groups were given single and double rings of ALC, respectively. Sampling surveys were carried out in the surf zone where released fish were densely distributed on 7 or 8 days (d) after release (Fig. 1). Japanese flounder juveniles were caught by beam trawl (1.5 m x 0.5 m) at 3 hour intervals over a 24 hour period. The beam trawl was towed by manpower. All fish were frozen in the field by using dry ice. Food organisms of juvenile flounder were also collected simultaneously by small beam trawl (0.5 m x 0.4 m).

Total length (mm), body weight (0.01 g) and body weight excluding the viscera (0.01 g) were measured. Stomachs were excised and preserved in 5% formalin for later analysis. The total stomach contents were weighed to the nearest milligram after blotting with filter paper and prey items were identified to the lowest possible taxonomic level and counted under microscope. Wet weights of the prey items were recorded to the nearest milligram. Food organisms collected by small beam trawl were also analyzed as the stomach contents.

Estimation of Daily Ration

Daily ration was estimated in terms of percent of BW from the model of Elliott and Persson (1978):

$$Ct = (St - S0 \, EXP \, (-R \times t \,)) \times R \times t \, / \, (1 - R \times t), \qquad (1)$$

where Ct is the consumption of food during the time interval (t), $S0$ and St are the average stomach contents index (SCI : stomach contents weight x 100/BW) at time 0 and t, respectively, and R is the instantaneous evacuation rate. The estimates of Ct calculated for each time interval are then summed to give the total daily ration. Feeding is assumed constant within each time interval.

In the laboratory experiment under natural photoperiod condition, even though Japanese flounder juveniles can feed on live mysids ad libitum, the average SCI of flounder clearly decreased during nighttime (Tominaga and Kawai, unpublished data). Assuming no feeding between sunset and sunrise, the instantaneous evacuation rates were estimated from the depletion of stomach content index during night (including empty stomachs). Evacuation rate is therefore given by:

$$R = (1 / t') Ln(Smax / Smin),$$ (2)

where the instantaneous evacuation rate is calculated from the maximum ($Smax$) and minimum ($Smin$) average SCI of the sample collected during night and the time interval between $tmax$ and $tmin$ (t').

Daily ration of the wild flounder was estimated only in July 1997, because the size of wild fish collected in the field is much smaller than that of hatchery-reared fish collected before July.

Laboratory Experiment of Stable Isotope

The rearing experiment was conducted at the Ocean Research Center of Fukui Prefectural University in May 1999. A total of 228 juveniles (average TL of 47.5 mm) to which formula feed had been fed for two weeks were divided into two groups on 16 May 1999. Seventy-eight individuals were transferred to 30-L polycarbonate tank and reared by the same formula feed (control group). The remaining 150 individuals were reared by live mysids (*Archaeomysis* sp.) in 200-L polyethylene tank for 2 weeks (experiment group). Mysids were caught in Obase Beach Wakasa Bay twice a week and preserved in a 500-L polycarbonate tank. Enough live mysids were added to the tank every day as the juveniles could prey on them ad libitum. Five individuals from the 30-L tank were sampled at 6 and 13 d after the beginning of the experiments and 5 individuals from the 200-L tank were randomly collected every two days during the experiment.

The fish were kept frozen until analyzing. Total length and body weight of individual fish were measured. The dorsal muscle on the eye side was excised, dried, ground to a powder and preserved in a chloroform-methanol solution to remove lipids. The powdered sample (0.5-1.0 mg) was put in a tin container. The carbon isotope ratio ($\delta^{13}C$) was analyzed by a Mat Delta S staple isotope ratio mass spectrometer (Finnigan MAT) equipped with an EA-1108 elemental analyzer (Carlo Erba) at the Center for Ecological Research, Kyoto University.

Since the variation of stable isotope ratios is so small, the isotopic compositions are described by a per mil (‰) deviation from each international standard as defined by the following equation:

$$\delta^{13}C = (Rsample / Rstandard - 1) \times 1000,$$ (3)

where R denotes $^{13}C/^{12}C$. A fossil calcium carbonate was used as the standard. Then, DL-Alanine was used as a laboratory secondary standard to insure instrument accuracy and precision. Instrumental precision was 0.1 ‰.

We fitted our data to Hesslein's model (Hesslein *et al.,* 1993):

$$\delta^{13}C = A + (B - A) \times exp(-C \times t),$$ (4)

where *A* and *B* are parameters determined by asymptotic and initial conditions, respectively, *C* is the rate of change in $\delta^{13}C$ related to growth rate and turnover rate of carbon in the tissue and t is time (day) since the diet switch. Each parameter was estimated by the maximum-likelihood method with STATISTICA (THREE'S COMPANY, INC.).

Results

Biomass of Food Organisms

An average biomass of both total food oraganisms and mysids collected with the small beam trawl net was highest in May 1997 and lowest in July 1997, (Table 2). The biomass of mysids in May, which was a main diet of Japanese flounder juveniles, was 11 times that in July. The biomass was an intermediate value in May 1998. The dominant genera of mysids were *Arcaheomysis* and *Nipponomysis* in all surveys.

Table 2. Average biomass of total food organisms and mysids sampled at 24-hour surveys in Wada beach

	1997		1998
	5.Jun	9.Jul	29.May
Average biomass g/m²			
Total food organisms	0.36	0.08	0.17
Mysids	0.22	0.02	0.07

Stomach Contents

Mysids were the most important prey item in all surveys. Mysids were especially dominant in stomachs in June 1997 when mysids were the most abundant in the environment (Fig. 2). In May 1998, stomach contents of both large and small groups were similar and mainly composed of mysids, but also amphipods (Fig. 3). Stomach content composition of the late group in 1997 was similar to that of wild juveniles collected together (Fig. 2). Japanese anchovy, amphipods and maclura were also important food items.
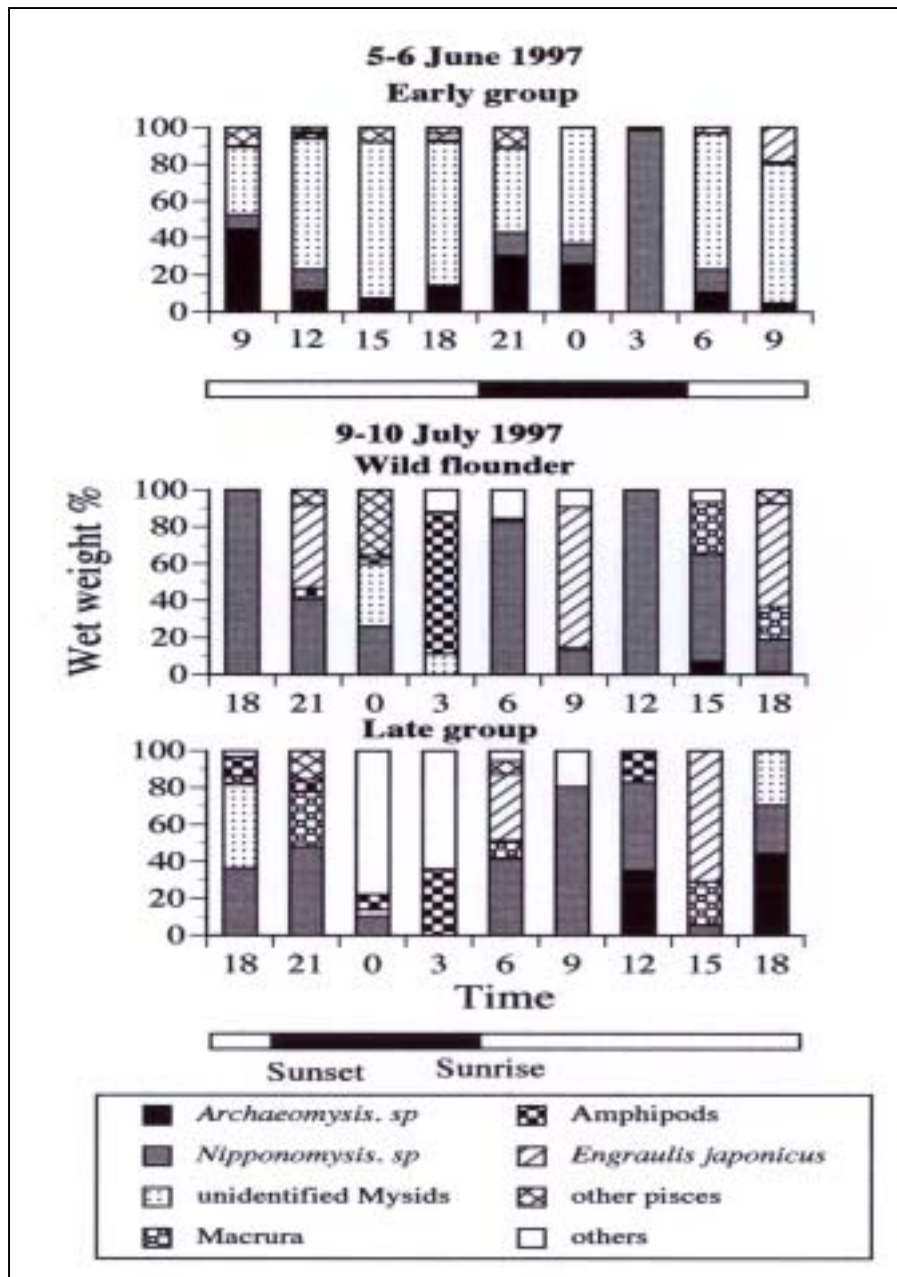
Figure 2. Diel changes in diet composition (wet weight %) in stomachs of hatchery-reared and wild *Paralichthys olivaceus* juveniles in June and July 1997. Open and closed bars indicate daytime and nighttime, respectively.

Figure 3. Diel changes in diet composition (wet weight %) in stomachs of hatchery-reared *Paralichthys olivaceus* juveniles in May 1998. Open and closed bars indicate daytime and nighttime, respectively.

Feeding Periodicity and Daily Ration

The average SCIs of flounder juveniles collected in 24 hour surveys were high during day time (Fig. 4). The clear peak of SCI was usually found around dusk and/or dawn, except for the late group in 1997 (Fig. 5). The average SCI of fish in the late group was constantly low throughout the day. However, it was common that the depletion of SCI was seen during nighttime. In 1998 the SCIs of wild flounder juveniles were constantly higher than those of the late group during the day (Fig. 4).
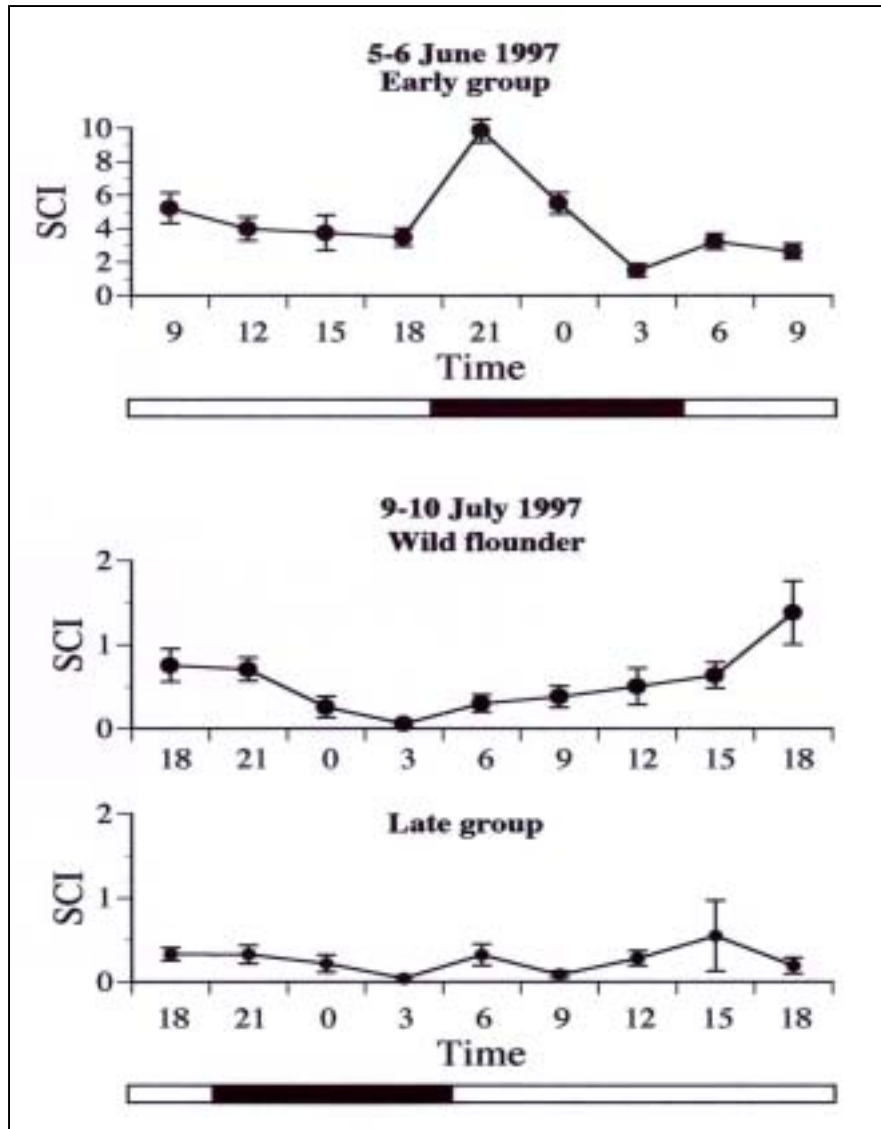
Figure 4. Diel changes in mean stomach contents index (stomach contents weight x 100/body weight) of hatchery-reared and wild *Paralichthys olivaceus* juveniles in June and July 1997. Vertical lines indicate standard error. Open and closed bars indicate daytime and nighttime, respectively.
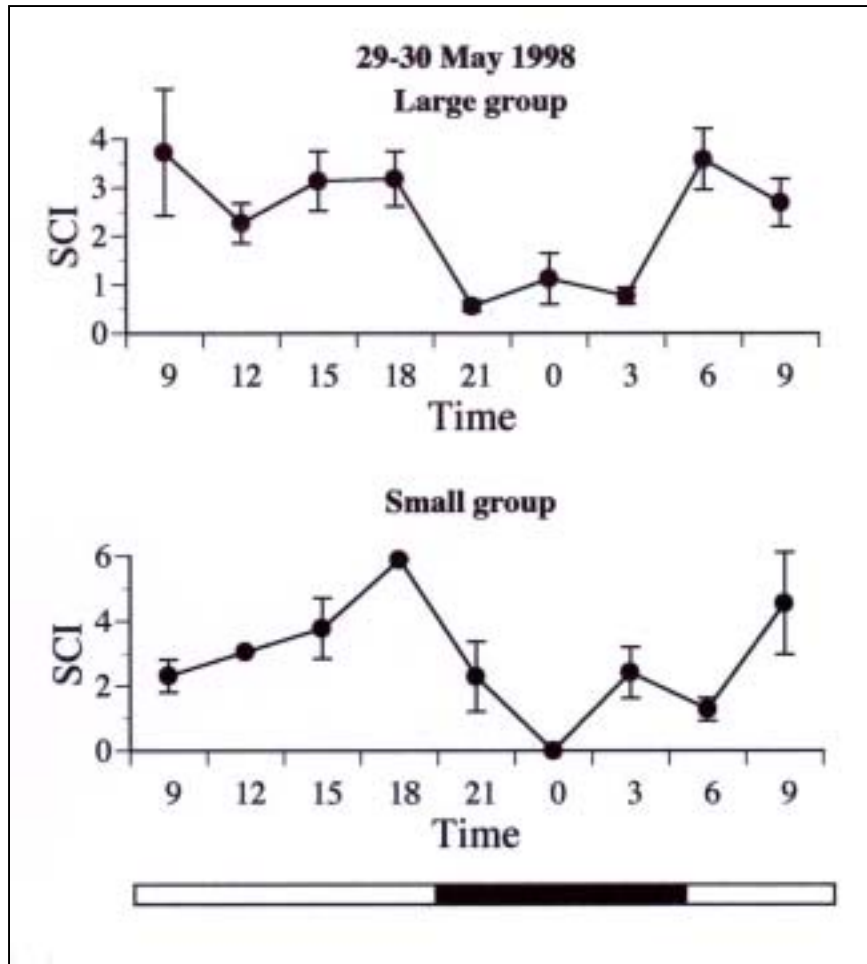
Figure 5. Diel changes in stomach contents index (stomach contents weight x 100/body weight) of hatchery-reared *Paralichthys olivaceus* juveniles in May 1998. Vertical lines indicate standard error. Open and closed bars indicate daytime and nighttime, respectively.

Instantaneous evacuation rate (R) was estimated by using equation (2). The evacuation rate estimate of hatchery-reared fish was similar (0.28-0.31) in all surveys (Table 3). However, R (0.4) of wild flounder was much higher than that of hatchery-reared fish.

The average SCI, which included fish with empty stomachs, was used to estimate the average food consumption for each time interval (Ct) with equation (1). Food consumption per unit time (1 hour) presented negative, as well as positive, values. Daily ration was obtained by summing the amount of food consumption during each interval, including negative values.

Diel change in food consumption per unit time was similar to that of the SCI. The feeding intensity of released flounder in May 1997 and wild flounder in July 1997 gradually increased after sunrise and peaked around dusk (Fig. 6). However, the average food consumption per unit time of fish released in July 1997 was constantly low for 24 hours and clear feeding periodicity was not found. There were two peaks of feeding around dusk and dawn in 1998 (Fig.7).
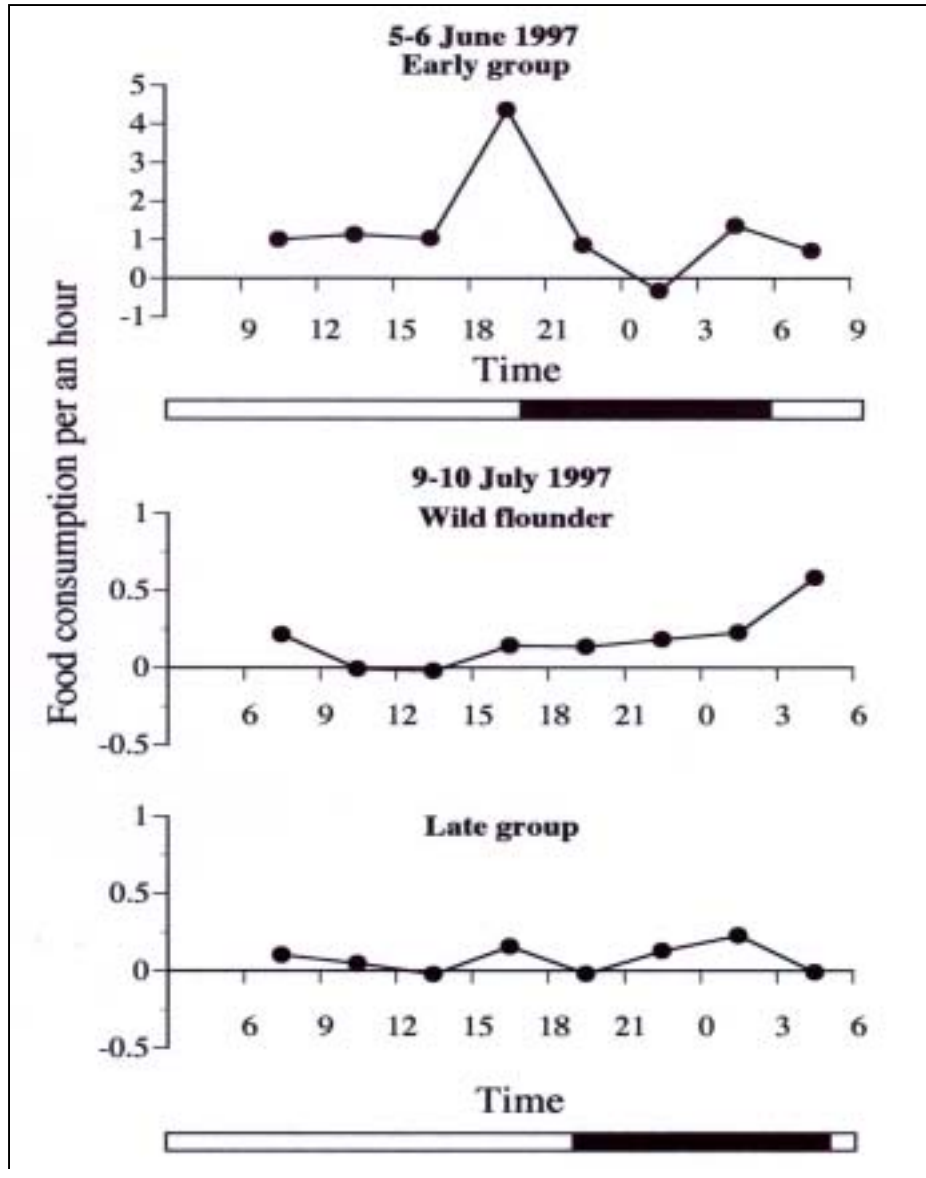
Figure 6. Diel changes in mean food consumption per an hour of hatchery-reared and wild *Paralichthys olivaceus* juveniles in June and July 1997. Open and closed bars indicate daytime and nighttime, respectively.
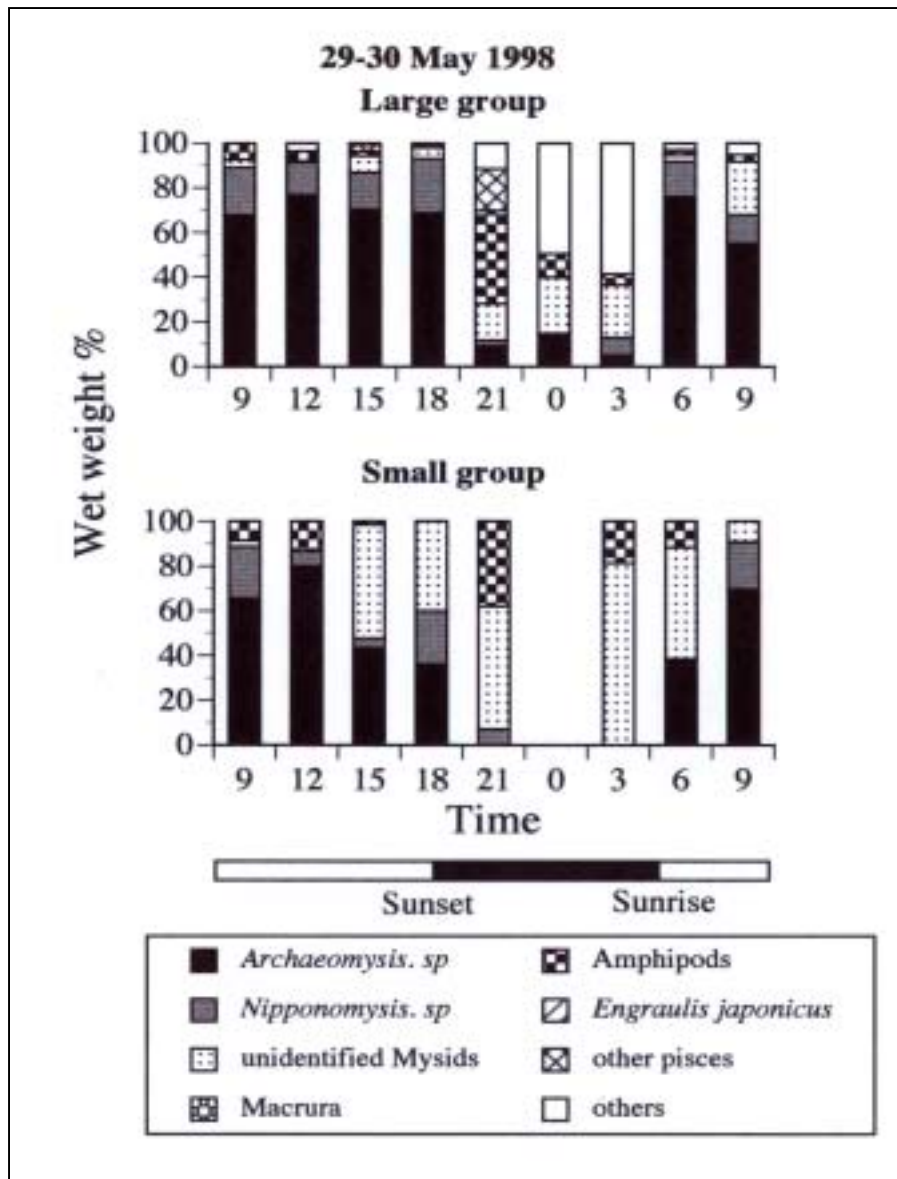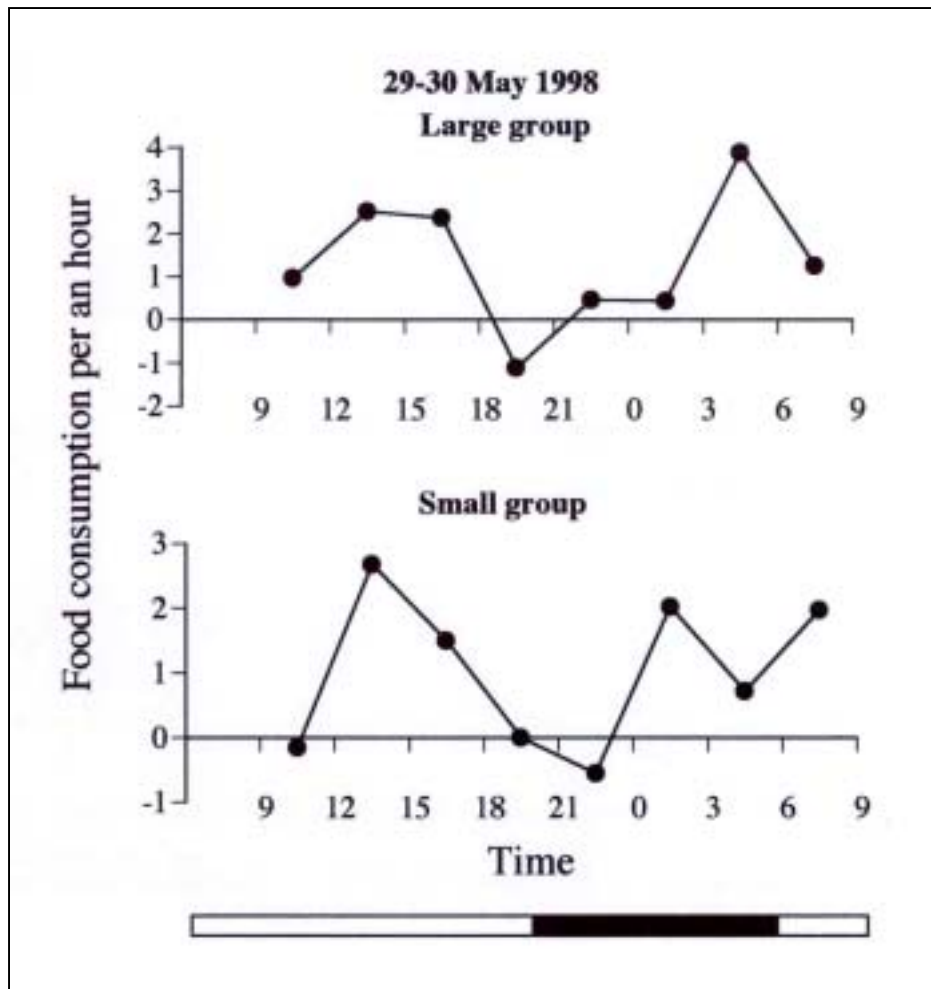
Figure 7. Diel changes in mean food consumption per hour of hatchery-reared *Paralichthys olivaceus* juvenile in May 1998. Open and closed bars indicate daytime and nighttime, respectively.

The estimated daily ration was the highest in May 1997 (22.2 %/BW/d, Table 3). In 1998, daily ration of the large and small groups was 10.8%/BW and 8.2%/BW, respectively. The minimum value (1.5%/BW) of daily ration was found in July 1997. Daily ration (4.8%/BW) of wild flounder collected in July 1997 was 3 times that of hatchery-reared fish (Table 3).

Table 3. Instantaneous evacuation rate (R) and daily ration of released and wild Japanese flounder
Paralichthys olivaceus, and data about 24-hour field surveys in 1997 and 1998.

	1997	1997		1998	
	Early group	Late group	Wild fish	Large Group	Small group
Date of survey	5.Jun	9.Jul		29.May	
Average TL collected	46.6mmTL	50.7mmTL	55.9mmTL	53.3mmTL	38.4mmTL
Average No. of fish collected during night time (No. /5 min tow)	49.7	10.3	30.9	19.1	3.8
R-value	0.29	0.31	0.40	0.29	0.28
Daily ration %BW/day	22.2	1.5	4.8	10.8	8.2
Water temperature	20℃	24℃		21℃	

The Experiment of Stable Isotope

The initial average body weight of fish used in this experiment was 1.06 g (0.46 SD). The body weight at the end of the experiment was 3.80 g (0.69 SD) for the experiment group and 2.95 g (1.37 SD) for control group. The daily growth rate (g/day) of the experimental group and control groups was 0.20 and 0.15, respectively.

The average $\delta^{13}C$ for the formula feed was -21.09‰ and -19.03‰ for the mysids, a difference of 2.06‰ (Fig. 8). The average $\delta^{13}C$ in the dorsal muscle was not significantly different among control groups sampled at 0, 6, and 13 d after the beginning of experiment (ANOVA). The average $\delta^{13}C$ of the control group was 20.67‰ and slightly more than the formula feed. However, the average $\delta^{13}C$ of the experimental group gradually increased from -20.70‰ to -19.19‰ (Fig. 8). The average $\delta^{13}C$ at the end of the experiment was slightly smaller than the mysids. A significant difference was found between the initial $\delta^{13}C$ and experimental group collected on 2 d (Tukey HSD test p = 0.013).

Our data were fitted to equation (4): $\delta^{13}C = -18.97 - 1.73\ exp\ (-0.14 \times t)$.
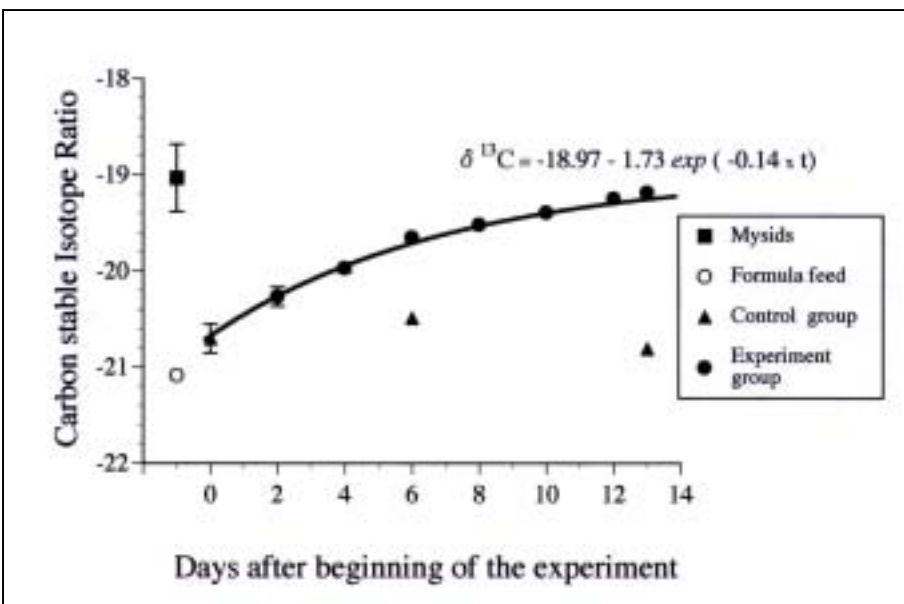
Figure 8. Changes in mean carbon stable isotope ($\delta^{13}C$) in the dorsal muscle of the control group fed formula feed and the experimental group fed live mysids. The $\delta^{13}C$ of mysids collected in Wakasa Bay and formula feed are plotted. Hesslein's model, $\delta^{13}C = -18.97 – 1.73\ exp\ (-0.14xt)$, was fitted to the data of the experimental group. Vertical lines indicate standard error.

Discussion

Daily Ration as an Indicator Evaluating Release Tactics

Japanese flounder juveniles prey mainly on mysids in the nursery area (Yasunaga and Koshiishi, 1981; Kato, 1987; Furuta *et al.*, 1997; Tanaka *et al.*, 1999). In the present study, mysids were the major food item for the released flounder. However, stomach contents composition fluctuated according to mysid biomass. Although mysids composed more than 90% of the diet in May 1997 when the mysids were the most abundant, the diversity of diet composition in the stomach increased as the mysid biomass decreased. Tanaka *et al.* (1999) pointed out that the early shift in diet from mysids to fish was found in this study area comparing to other areas (Tane, 1992; Fujii and Noguchi, 1996) and suggested that mysid availability was relatively low. The biomass of mysids fluctuates seasonally and yearly at Wada beach and the variation of stomach contents composition seems to reflect the food availability for released fish.

Daily ration of released fish was also closely related to the food availability, especially mysid biomass. Daily ration of the early group in 1997 was 14.8 times that of the late group. The catch rate of released flounder in fishing surveys decreased rapidly during the first several days (Tominaga, 1991; Furuta *et al.*, 1997) and predation is thought to be a major cause of early mortality (Sudo *et al.*, 1992; Yamashita *et al.*, 1993; Furuta *et al.*, 1998). However, Furuta (1998) reported that a short-term starvation (3 to 7 days) changed feeding behavior of flounder juveniles and resulted in the vulnerability to predation. It is reasonable to think that feeding conditions of released fish indirectly affect early mortality after release. An average number of early group fish caught during nighttime (49.7 individuals/5 minute tow) was about 5 times as much as that of late group (10.3 inds/5min. tow). A large reduction of released flounder (including an emigration from release area) was found in the late group, where feeding intensity was low.

Food intake of released flounder was lower and incidence of empty stomachs were higher than wild flounder in Tottori (Furuta *et al.*, 1997). In the present study, the daily ration of wild flounder in July 1997 was 3 times that of the late group. These results show that hatchery-reared fish are inferior predators. Predation ability is necessary for hatchery-reared fish seedling quality and survival.

It is possible to compare daily ration of fish collected in different places and/or at different times. Daily ration is thought to reflect food availability in the release area and seedling quality, and be used as an indicator to evaluate the quality of the release technique. However, to evaluate the actual effect of release, sampling surveys of commercial landings are indispensable.

Possibility of New Methods by Stable Isotope

Carbon has stable isotope, ^{12}C and ^{13}C. Every biogenic substance and living organism in an ecosystem consists of these isotopes (Wada *et al.*, 1998). Stable isotopic compositions of consumer tissues can often be related to stable-isotopic compositions of diet (DeNiro and Epstein, 1978). Therefore, stable isotope (especially carbon and nitrogen) has been used in the ecological study of energy flow (e.g. Hobson and Welch, 1995; Wainwright *et al.*, 1993).

In the present study we tried to apply the carbon stable isotope to the quantitative study of diet consumed by hatchery-reared flounder. When the diet was switched from formula feed to live mysid, $\delta^{13}C$ in the dorsal muscle of the experimental group began to approach $\delta^{13}C$ of mysids. In the Hesslein's model, coefficient "*C*" indicates the magnitude of the rate of change in $\delta^{13}C$. This value is associated with carbon turnover rate and growth rate of fish. The growth rate

of juveniles used in this experiment is extremely high. Average BW changed from 1.06 g in the beginning to 3.80 g at the end of the experiment (14 d). There are few studies on turnover rate of carbon isotope in fish tissue (Matsubara, 1997). Hesslein *et al.* (1993) examined the change in the isotope composition of carbon in broad whitefish (*Coregonus nasus*) tissues in response to a change in the isotope composition of their food and showed that turnover rate of carbon isotope was very small.

In the present experiment, it is thought that change in $\delta^{13}C$ is mainly attributed to fish growth rate. In Japanese flounder juveniles, the rate of change in $\delta^{13}C$ would directly reflect the growth rate. Therefore it would be possible to estimate the growth rate and consequently the cumulative food consumption after release by the rate of change in $\delta^{13}C$. However, experiments of different amounts of food and mixtures of diet with different stable isotope ratios must be conducted.

Acknowledgments

Literature Cited

Cochran, P. A. 1979. Comments on some recent methods for estimating food consumption by fish. *J. Fish. Res. Board Can.* 36:1018.

DeNiro M. J. and S. Epstein. 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochemica et Cosmochimica Acta* 42:495-506.

Eggers, D. M. 1979. Comments on some recent for estimating food consumption by fish. *J. Fish. Res. Board Can.* 36:1018-1019.

Elliott, J. M. 1979. Comments on some recent methods for estimating food consumption by fish. *J. Fish. Res. Board Can.* 36:1020.

Elliott, J. M. and L. Persson. 1978. The estimation of daily rates of food consumption for fish. *J. Anim. Ecol.* 47:977-991.

Fujii T. and M. Noguchi. 1996. Feeding and growth of japanese flounder (Paralichthys olivaceus) in the nursery ground, pp. 141- 151, In Y. Watanabe, Y. Yamashita,and Y. Oozeki (eds.), Survival Strategies in Early Life Stages of Marine Resources, A.A. Balkema, Rotterdam.

Fujita T., T. Mizuno, and Y. Nemoto. 1993. Stocking effectiveness of Japanese flounder *Paralichthys olivaceus* fingerling released in the coast of Fukushima prefecture. *Saibai Giken* 22:67-73. (in Japanese).

Furuta S., T. Watanabe, H. Yamada, and T. Miyanaga. 1997. Changes in feeding condition of released hatchery-reared Japanese flounder *Paralichthys olivaceus* and prey mysid density in the coastal area of Tottori Prefecture. *Nippon Suisan Gakkaishi* 63:886-891.

Furuta S., T. Watanabe, H. Yamada, T. Nishida, and T. Miyanaga. 1997. Changes in distribution, growth and abundance of hatchery-reared Japanese flounder *Paralichthys olivaceus* released the coastal area of Tottori Prefecture. *Nippon Suisan Gakkaishi* 63:877-885.

Furuta S. 1998. Effects of starvation on feeding behaviour and predation vulnerability of wild Japanese flounder juveniles. *Nippon Suisan Gakkaishi* 64:658-664.

Furuta S., T. Watanabe, and H. Yamada. 1998. Predation by fishes hatchery-reared Japanese flounder *Paralichthys olivaceus* juveniles released the coastal area of Tottori Prefecture. *Nippon Suisan Gakkaishi* 64: 1 - 7.

Hesslein R. H., K. A. Hallard, and P. Ramlal. 1993. Replacement of sulfur, carbon and nitrogen intissue of growing broad whitefish (*Coregonous nasus*) in response to a change in diet traced by δ^{34}S, δ^{13}C, and δ^{15}N. *Can. J. Fish. Aquat. Sci.* 50:2071-2076.

Hobson K. A. and H. E. Welch. 1995. Cannibalism and trophic structure on a high Arctic lake: insights from stable isotope analysis. *Can. J. Fish. Aquat. Sci.* 52:1195-1201.

Kato K. 1987. Study of resources management of flounder, *Paralichthys olivaceus* in the northern coast of Niigata. 1. Distribution and food habit o juvenile flounders. *Bull. Niigata Pref. Fish. Exp. Sta.* 12:27-41.

Matsubara T. 1997. Availability and problem o stable isotope in food habit analysis of animals: For example avian and benthic animals. *Kaiyo Monthly* 29:425-429.

Sudo H., T. Goto, R. Ikemoto, M. Tomiyama, and M. Azeta. 1992. Mortality of reared Flounder (*Palalichthys olivaceus*) juveniles released in Shijiki Bay. *Bull. Seikai Natl. Fish. Res. Inst.* 70:29-37.

Tanaka Y., O. Tominaga, T. Tsusaki, K. Nogami, H. Fushimi, and M. Tanaka. 1999. UJNR experimental release of Japanese flounder at Awda beach, Wakasa Bay : Feeding habits of wild and released juveniles. *Bull. Natl. Res. Inst. Aquacult., Suppl.* 1:125-133.

Tane S. 1992. Feeding ecology of Japanese flounder (*Paralichthys olivaceus*) Japanese flounder juvenile in Yura estuary, Kyoto. MS thesis Kyoto University, pp, 1 - 92.

Tominaga O. 1991. Tagging experiment of hatchery-reared Japanese flounder, Tech. *Rep. Hokkaido Natl. Fish. Res. Inst.* 2:27-30.

Tominaga O. and Y. Watanabe. 1998. Geographical dispersal and optimum release size of hatchery-reared Japanese flounder *Paralichthys olivaceus* released in Ishikari Bay, Hokkaido, Japan. *Journal of Sea Research* 40:73-81.

Wada E., I. Tayasu, K. Koba, T. Matsubara, N. O. Ogawa, Y. Yamada, K. Yoshii, and A. Sugimoto. 1998. In: The use of stable isotopes for ecological studies, pp. 407-430, Gopal B., P. S. Pathak and K. G. Saxena (eds.), Ecology Today: An Anthology of Contemporary Ecological Research, International Scientific publications, New Dehhi.

Wainwright S. C., M. J. Fogarty, R. C. Greenfield, and B. Fry. 1993. Long-term change in the George bank food web: trends in stable isotopic compositions of fish scales. *Mar. Biol.* 115:481-493.

Yamashita Y., K. Yamamoto, S. Nagahora, K. Igarashi, Y.Ishikawa, O.Sakuma, H. Yamada, and Y. Nakamoto. 1993. Predation by fishes on hatchery-raised Japanese flounder, *Paralichthys olivaceus*, fry in the coastal waters of Iwate prefecture, northeastern Japan. *Suisanzosyoku* 41:497-505.

Yamashita Y., S. Nagahora, H. Yamada, and D. Kitagawa. 1994. Effects of release size on survival and growth of Japanese flounder *Paralichthys olivaceus* in coastal waters off Iwate prefecture, northeastern Japan. *Mar. Ecol. Prog. Ser.* 105:269-276.

Yasunaga Y. and Y. Koshiishi. 1981. Basic studies of problem on the propagation of plaice, *Paralichthys olivaceus*, 2. A consideration of the relation between distribution density of juvenile plaice and mysids. *Bull. Japan Sea Reg. Fish. Res. Lab.* 32:9-26.

Nucleic Acids and Protein Content as a Measure to Evaluate the Nutritional Condition of Japanese Flounder *Paralichthys olivaceus* Larvae and Juveniles

Woo-Seok Gwak
The University of Georgia Marine Institute
Sapelo Island, Georgia 31327
USA
Email: gwakws@arches.uga.edu

Abstract

Recently developed fluorescence techniques were used to quantify RNA and DNA content in the whole body of fed and starved hatchery-reared larval and juvenile Japanese flounder. RNA/DNA ratios in wild larvae and juveniles were concurrently measured to evaluate their nutritional condition. Significant differences in the RNA/DNA ratios were found between fed and starved fish, and appeared to expand drastically as starvation proceeded. Even in the fed fish marked fluctuations in its ratios during metamorphosis were observed, evident by decreasing from late metamorphic to post-metamorphic stages. The changes in protein content coincided well with RNA content. Protein/DNA ratio also peaked at the post-metamorphic phase and decreased for several days thereafter, suggesting an occurrence of hypertrophy until the post-metamorphic phase followed by hyperplasia. Using the criteria established from these laboratory experiments, the nutritional conditions of wild Japanese flounder larvae and juveniles collected in Wakasa Bay in 1994 and 1995 were determined by measuring RNA and DNA. Starved fish were mainly found at stage I (settling stage) fish during the late season of settlement in 1995. The present study demonstrates the usefulness of RNA/DNA ratios and protein content for assessment of the nutritional condition of hatchery-reared and wild Japanese flounder larvae and juveniles.

Japanese flounder *Paralichthys olivaceus* is a commercially important species for mariculture and stock enhancement as well as coastal fisheries in Japan. Although a large variety of work has been done on the early life stages of Japanese flounder (Minami, 1982; Seikai *et al.*, 1986; Fukuhara, 1986), less is known about the mechanisms of its mortality in the sea (Tanaka *et al.*, 1989a). Hjort (1914) first proposed the critical period hypothesis, whereby the year class size in most marine fish species hatched from small pelagic eggs could be determined primarily from high mortality rates during early life stages. Consequently, small changes of survival rate during this time can give rise to high variability in recruitment (Sale, 1990; Fogarty, 1993). Over the years, laboratory studies have mainly focused on mortality during the first feeding stage of marine fish larvae (May 1974). More recently, however, several papers have begun to address the metamorphic phase of fish development as a critical period for survival (Thorisson, 1994). Recent works in flatfish particularly have tried to elucidate the possibility of a second severe mortality event during the post-metamorphic phase of settlement (Tanaka *et al.*, 1989a; Phil, 1990; Keefe and Able, 1993). Thus, the early larval stage would be a crucial period for survival and potential occurrence of another species-specific critical period can be predicted in association with metamorphosis in the Japanese flounder.

A variety of techniques such as morphometric, histological, and biochemical analyses for diagnosing the nutritional condition of fish larvae and juveniles have been developed and applied to both hatchery-reared and wild fish (Buckley, 1979; Yin and Blaxter, 1986; Clemmesen, 1987; 1988; Theilacker and Watanabe, 1989). Biochemical analysis, which determines the quantities of chemical constituents that serve as energy substrates, could be one of the indicative measures to show changes of nutritional conditions. Among the biochemical indices, the ratio of RNA to DNA has been proven a reliable indicator of nutritional condition (Buckley, 1979; 1980) and growth of larval and juvenile fish (Bulow, 1970). Tanangonan *et al.* (1998) did preliminary studies of biochemical changes for hatchery-reared Japanese flounder. However, more detailed experiments are needed to assess nutritional condition of Japanese flounder associated with development and apply the biochemical criterion to the wild fish.

In the present study the RNA, DNA, and protein content of wild and hatchery-reared larval and juvenile Japanese flounder are investigated to evaluate nutritional condition and the usefulness of such techniques.

Materials and Methods

Japanese flounder larvae and juveniles were reared at the Fisheries Research Station of Kyoto University, Maizuru, Japan, in April and May 1994. Fertilized eggs, provided by the Japan Sea Farming Association (Miyazu Station), were stocked into 500-L polycarbonate tanks, and maintained at 15 C using running seawater. Within six hours of stocking, water temperature was raised to an average of 18 C using heating rods connected to a thermostat. Larval rearing was conducted under natural photoperiod conditions. The larvae were fed rotifers (*Brachionus plicatilis*) cultured with *Nannochloropsis oculata* at three days after hatching (DAH), and brine shrimp (*Artemia salina nauplii*) enriched with squid liver oil at 20 DAH. Rotifers were provided at a density of five ml^{-1} until 28 DAH, and brine shrimp were given until 43 DAH at a density of one to six ml^{-1} every morning.

In Experiment (Exp.) 1 RNA/DNA ratios showed large fluctuations at stages G, H, and I due to failure in determining the sample amount from the homogenized one. In order to confirm the values of RNA/DNA ratios at specific stages during metamorphosis, a supplemental, second experiment was performed under almost identical rearing conditions as the first. Experiment 2 lasted for 15 days between the early-metamorphosing stage (F) and eight days after settlement (I_8).

To reconfirm the changes in RNA/DNA ratios during metamorphosis and settlement, a rearing experiment lasting for 21 days from E to I_8 stage (eight days after reaching I stage) was designed and carried out under similar rearing conditions to the previous experiments. To initially reduce individual variance, approximately 3,000 larvae at E stage were carefully selected from the 500-L stocking tanks and transferred into 100-L rearing tanks. Development of Japanese flounder larvae and juveniles was classified into nine developmental stages according to Minami (1982), and its metamorphic process was grouped into three phases (Gwak *et al.*, 1999).

The starvation phase involved 300 fish at each developmental stage from A to E carefully sorted from the three 500-L stocking tanks during Exp.1 and transferred into 100-L tanks. Three hundred fish from the F stage were also selected during the second experiment and transferred into 100-L tanks. The fish were kept in circulated seawater with aeration at 18 C without food.

The starvation experiment ended on the day of 100% mortality based on the result of a previous point-of-no-return study by Gwak *et al.* (1999).

Samplings for fed and starved fish at each developmental stage were done during 43 days from 3 DAH for the fed group and just before the day of 100% mortality for the starved group (Table 1). To determine quantities of DNA and RNA in the entire body, six fish were individually sampled, rinsed, pipetted into Eppendorf micro vials, immediately frozen at -25 C, and stored at -87 C until analysis. All the samplings and preservations for RNA/DNA ratios were made from both fed and starving groups during the rearing experiment. In Exp. 2 the sampling and preservation was done in the same manner.

Table 1. Criterion established by RNA/DNA ratio for determining the nutritional conditions of Japanese flounder larvae and juveniles.

Stage	Body Length (mm)	Characters	Metamorphic Phases	Metamorphosing Stages	Nutritional Status Dying $\leq$ starving* $\leq$ healthy
A	3.6	First feeding	Premetamorphic		1.48 – 2.15
B	4.4				1.34 – 2.15
C	5.7				2.13 – 3.19
D	7.4				1.16 – 3.50
E	7.7	Metamorphosis	Metamorphic	Early-	1.56 – 4.03
F	8.3				1.20 – 2.52
G	9.3			Mid-	1.08 – 3.24
H	11.4			Late-	1.69 – 5.36
I	11.6	Settlement	Postmetamorphic		1.35 – 3.78

* The range of RNA/DNA ratio that can be classified as a starving.

To determine the quantities of RNA, DNA, and protein content in the whole body during the third experiment, ten additional fish were sampled and stored until later analysis. For measurements of dry weight individual fish were placed on pre-weighed pieces of foil and dried to a constant weight at 60 C. To obtain a precise value of dry weight, especially for the smallest larvae, freshly activated silica gel was placed in the balance to control for relative hygrometry. A minimal amount of time spent from removal from the desiccator to weighing was controlled strictly.

Field collections were performed in Wakasa Bay, Sea of Japan using a net 1.3 m in diameter and 0.33 mm mesh for the pelagic larvae and a beam trawl (2.0 m mouth width and 4.0 mm mesh) for the settled juveniles during the settling season from March to June in 1994 and 1995. Temperature and salinity were measured at each site with a Yellow Springs International (YSI) SCI Meter 33. A total of 187 larvae and juveniles (Table 2) were analyzed for their individual RNA/DNA ratios. Since tissues of fish larvae deteriorate quickly due to autolysis (Theilacker 1978), individual larvae and juveniles were stored on dry ice immediately after collection. To determine if these animals were in starving condition, RNA/DNA ratios were compared to the values determined from the larvae and juveniles starved under laboratory conditions shown in Table 1. The samples with RNA/DNA ratios below these values were considered "starving."

Table 2. Percentage of starving larvae and juveniles in each sampling date for 1994 and 1995.

Sampling Date	Total Catch	Stage	No.	No. of Starving	% Starving	Depth (m)	Temp ('C)
13 May 1994	36	C	11	2	18	20-40	17
		D	10	2	20		
		E	13	0	0		
		G	2	0	0		
20 May	39	B	4	0	0	20	17
		C	16	0	0		
		D	7	0	0		
		E	7	0	0		
		F	4	0	0		
		G	1	0	0		
6 April 1995	1	F	1	0	0	113	15
21 April	8	G	4	0	0	10	16
		H	2	1	50		
		I	2	1	50		
8 May	54	F	2	0	0	3.5-10	17
		G	18	0	0		
		H	19	0	0		
		I*	15	1	0		
25 May	34	I	34	22	65	4.5-5	17
5 June	15	F	2	0	0	3.5	18
		G	2	0	0		
		I	11	10	91		

* I stage fish that are newly or recently settled.

The quantity of RNA and DNA in the whole body from A to E stage was determined individually by using a specific nucleic acid fluorescent dye, Ethidium Bromide, developed by Clemmesen (1993), and slightly modified by Sato *et al.* (1995). In F stage, nucleic acids were extracted from the whole body by homogenizing the larvae and juveniles in Tris-HCl buffer (0.05 M Tris, 0.1 M NaCl, 0.01 M EDTA, pH 8.0), using a glass homogenizer placed in 4 C ice-cold water. In order to measure the DNA content of a sample, RNA was enzymatically digested with RNase and the remaining DNA was determined with Ethidium Bromide. Salmon sperm DNA (Wako Pure Chem. Co., Ltd.) and yeast RNA (Kanto Chem. Co., Ltd.) were used as standards.

A Bio-Rad protein kit, using bovine serum albumin as a standard determined total protein dissolved in NaOH. Expressed as µg of protein per fish, the ratio of RNA to protein and protein to DNA are cited as indices of protein synthesis capacity and cell size, respectively (Mathers *et al.*, 1994). A one-way ANOVA (Fisher) was used for statistical evaluation. Significance was accepted for *P*<0.05.

Results

Developmental Changes

        Figure 1 shows the developmental changes in RNA and DNA contents obtained from the Exp. 1. RNA and DNA contents of fed larvae increased with age, although the values largely fluctuated at G, H, and I stages. In Exp. 2, DNA contents increased gradually with age and showed a small peak just after settlement (on 34 DAH). The RNA content showed a drastic increase between the mid and late metamorphic stages (G to H), but almost no increment between early and mid metamorphic stages (F to G). The RNA content decreased between post-metamorphic phase (I stage) and 39 DAH, but repeatedly increased thereafter (Fig. 2).

Figure 1. Developmental changes in RNA and DNA contents in fed larvae and juveniles of laboratory-reared Japanese flounder. Data points are mean values of six fish. A to I indicate the developmental stage.

Figure 2. Ontogenetic changes in RNA and DNA contents of laboratory-reared Japanese flounder larvae and juveniles during metamorphosis (supplemental 2nd experiment). Data points are mean values of six fish. Vertical bars denote standard deviation, and F to I represent the developmental stage.

In Exp. 3, dry weight of larvae and early juveniles increased exponentially with development, enlarging individual variance in I stage (Fig. 3). These results indicate that changes in RNA and DNA content during metamorphosis were similar to those of Exp.2 (Fig. 4a). Ontogenetic changes in DNA and RNA content in terms of μg/mg DW (Fig. 4b) showed a very different pattern from those individually. The DNA content (μg/mg DW) at the early and mid stage appeared rather stable compared to the RNA content having an evident peak. Then both DNA and RNA contents showed a marked decrease until the post-metamorphic phase (I stage) before stabilizing again.
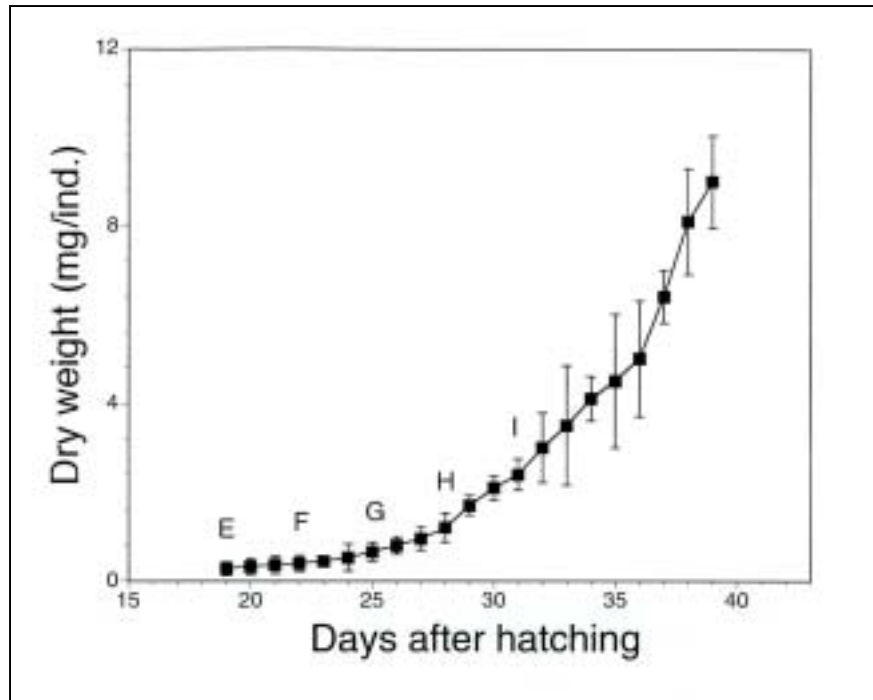
Figure 3. Developmental changes in dry weight (mg) of Japanese flounder larvae and juveniles during metamorphosis and early post-metamorphic phase (supplemental 3rd experiment). Values are given as the mean ± SD of twenty-five to thirty fish. E to I indicate developmental stage.

Figure 4a. Developmental changes in DNA and RNA contents of Japanese flounder larvae and juveniles during metamorphosis and early postmetamorphic phase (supplemental 3rd experiment) in :g/ind. (A) and :g/mg DW (B). Values are given as the mean ± SD of five to eight fish. E to I indicate developmental stage.
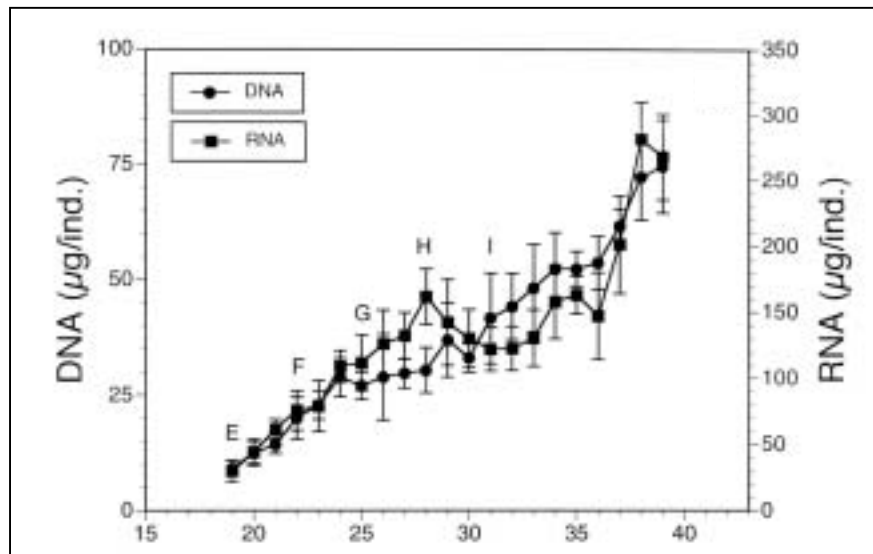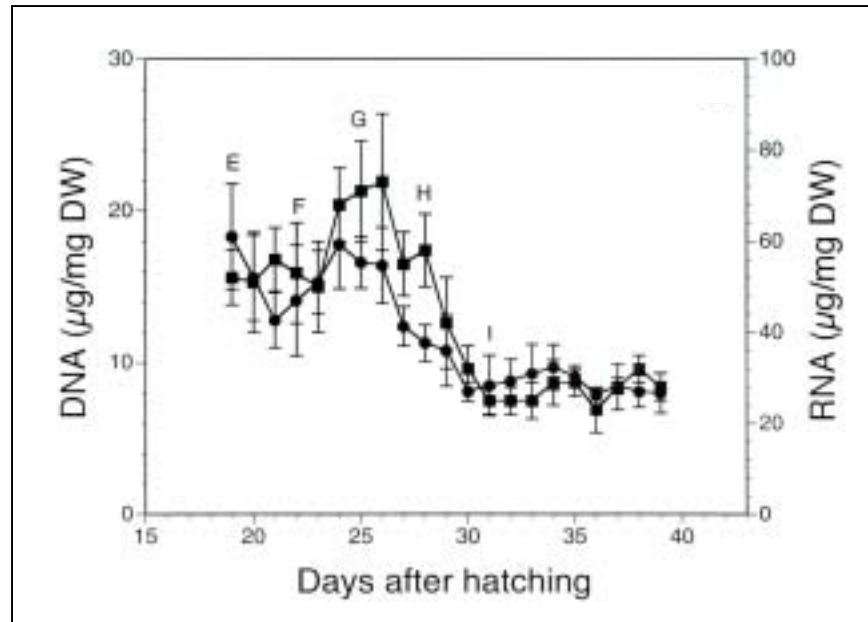
Figure 4b. Developmental changes in DNA and RNA contents of Japanese flounder larvae and juveniles during metamorphosis and early post-metamorphic phase (supplemental 3rd experiment) in :g/ind. (A) and :g/mg DW (B). Values are given as the mean ± SD of five to eight fish. E to I indicate developmental stage.

The protein content, as µg/ind., showed an overall increase between the early and late metamorphic stages, then stabilizing until reaching the I_2 stage (A in Fig. 5). After the post-metamorphic phase, the protein content of newly settled juveniles showed a drastic increase. A significant increase in protein content, as µg/mg DW, between the early and mid metamorphic stages was followed by a consistent decrease until the post-metamorphic phase (B in Fig. 5). Thereafter the protein content showed a gradual increase.

Figure 6 illustrates the relative amounts of DNA of the fed and starved larvae, with variability during development: lower in the fed larvae at D, E and H stages; higher at F, G, and I stages; and similar at A, B and C stages. The RNA content of the fed larvae exponentially increased with development, particularly accelerating at later phases of metamorphosis (G and H stages, Fig. 7). In contrast, the RNA contents in the starved larvae showed drastic reduction throughout the experiment with the onset of food deprivation. The rate of daily reduction appeared to be higher during the earlier days of starvation. Difference in RNA contents between fed and starved groups significantly increased as starvation proceeded, contrasting with DNA content that increased under starvation at several developmental stages.

Figure 5. Developmental changes in protein content of Japanese flounder larvae and juveniles during metamorphosis and early post-metamorphic phase (supplemental 3rd experiment) in :g/ind. (A) and :g/mg DW (B). Values are given as the mean ± SD of five to eight fish. E to I indicate developmental stage.

Figure 6. Developmental changes in DNA content of fed larvae and juveniles, and effects of starvation on the content in 1st (A) and 2nd (B) laboratory-reared Japanese flounder. Each point is the mean value of six fish. Vertical bars denote standard deviation, and A to I represent the developmental stage.
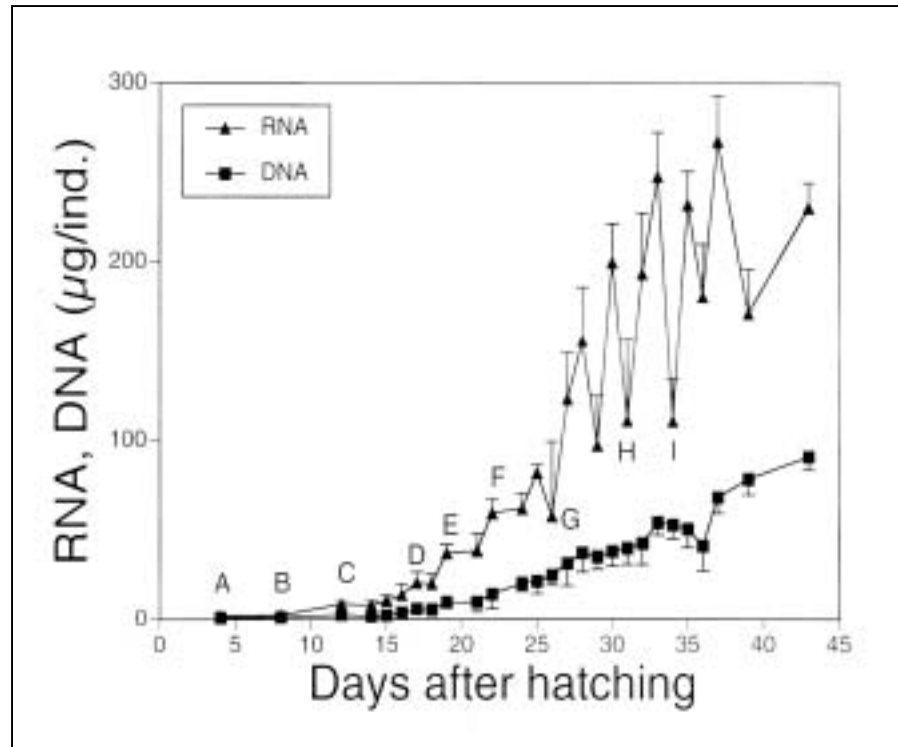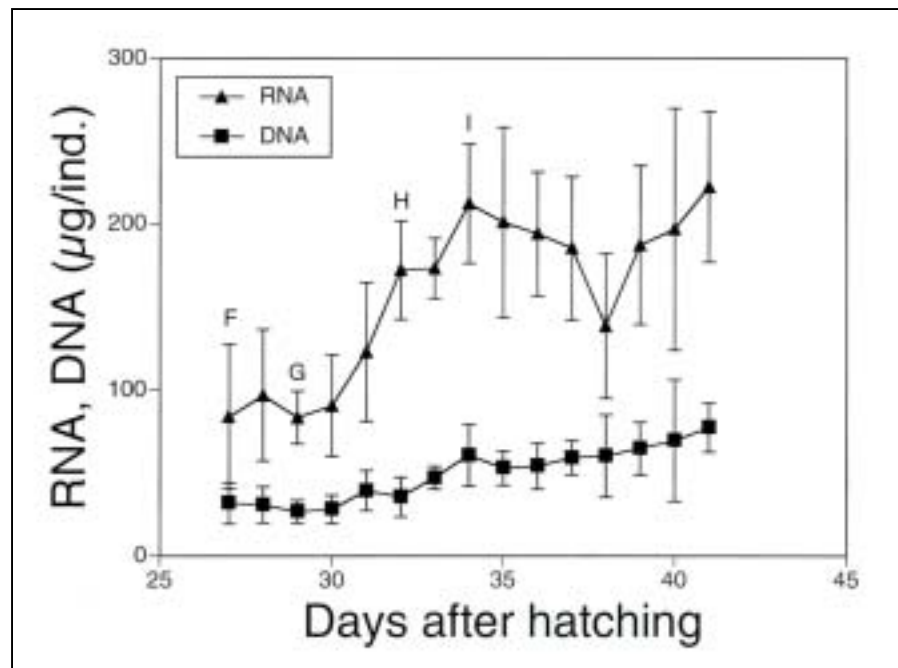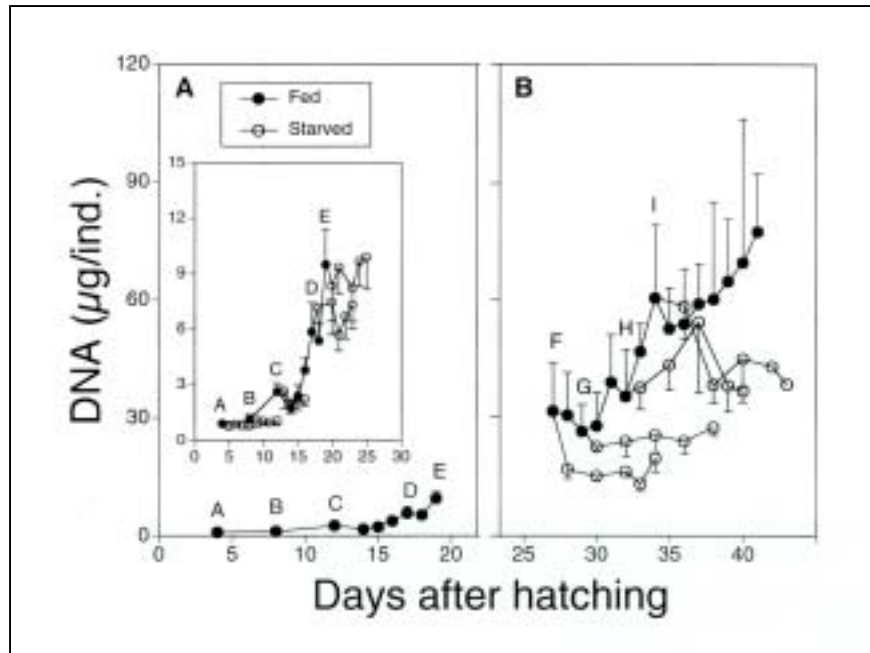

Figure 7. Developmental changes in RNA content of fed larvae and juveniles, and effects of starvation on the content in 1st (A) and 2nd (B) laboratory-reared Japanese flounder. Each point is the mean value of six fish. Vertical bars denote standard deviation, and A to I represent the developmental stage.
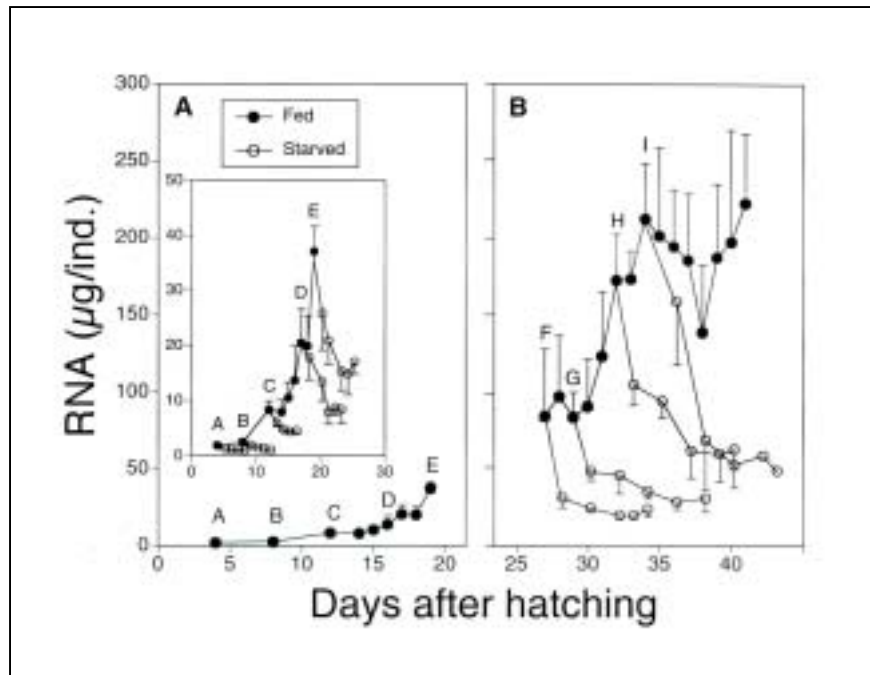
RNA: DNA, Protein: DNA, and RNA: Protein

The RNA: DNA for the fed group in Exp.1 showed, during the pre-metamorphic phase (A-E stages), a prominent increase between day 8 and 14, followed by a fluctuation until day 17 of D stage, and then reached a relatively steady level by day 22 of F stage (Fig. 8A). The RNA: DNA exhibited a marked fluctuation depending on developmental stage or day during the metamorphic (F to H) and post-metamorphic phases (I). The RNA: DNA obtained in Exp.2 showed a more stable pattern (Fig. 8B). It increased slowly between early and mid metamorphic stages (F and G), and reached the highest value of 5.36 ± 0.62 at the H stage. Thereafter, the value dropped to 2.49 ± 0.14 (I_5 stage: 5 days post-settlement), and then increased to 3.08 ± 0.25 at I_8. During larval starvation the ratio dropped consistently at every stage. For example, at the D stage it fell from 3.50 to 1.16 for five days, corresponding to 50% larval mortality (Fig. 8A).
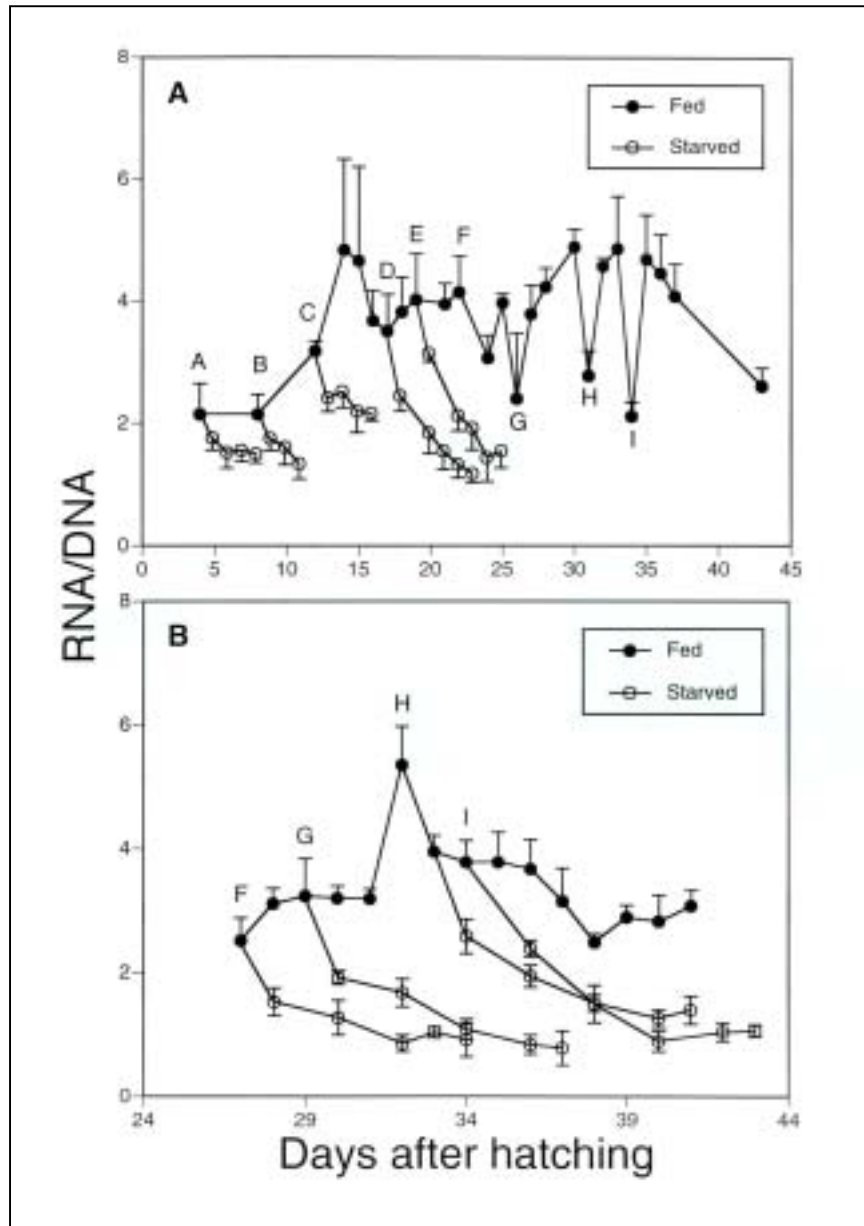
Figure 8. Developmental changes in RNA/DNA ratios of laboratory-reared Japanese flounder larvae and juveniles and changes in the ratios induced by starvation. A: 1st experiment, B: 2nd experiment. Vertical bars denote standard deviation, and A to I represent the developmental stage.

In Exp.3, RNA: DNA showed two peaks that occurred after onset of metamorphosis and at metamorphic climax stage H with the highest value of 5.07 ± 0.66 (Fig. 9). The RNA: DNA drastically dropped to 2.41 ± 0.85 at I_6 stage, and then tended to increase again. A gradual increase in DNA content versus a stable level of RNA content between late metamorphic and I_6 stages (Fig. 4A), caused an overall significant decrease in RNA: DNA. However, it increased again from I_6 stage with a relatively higher increase in RNA.

Protein: DNA also peaked at I stage resulting mainly from a higher rate of increase in protein content. The ratio then decreased markedly until the completion of metamorphosis, probably due to a slow increase in protein content, and a higher increase in DNA content (Fig. 10). Protein: RNA showed a drastic decrease at the early metamorphic stage and remained constant between mid and late metamorphic stages, followed by a slight decrease at post-metamorphic (Fig. 10).
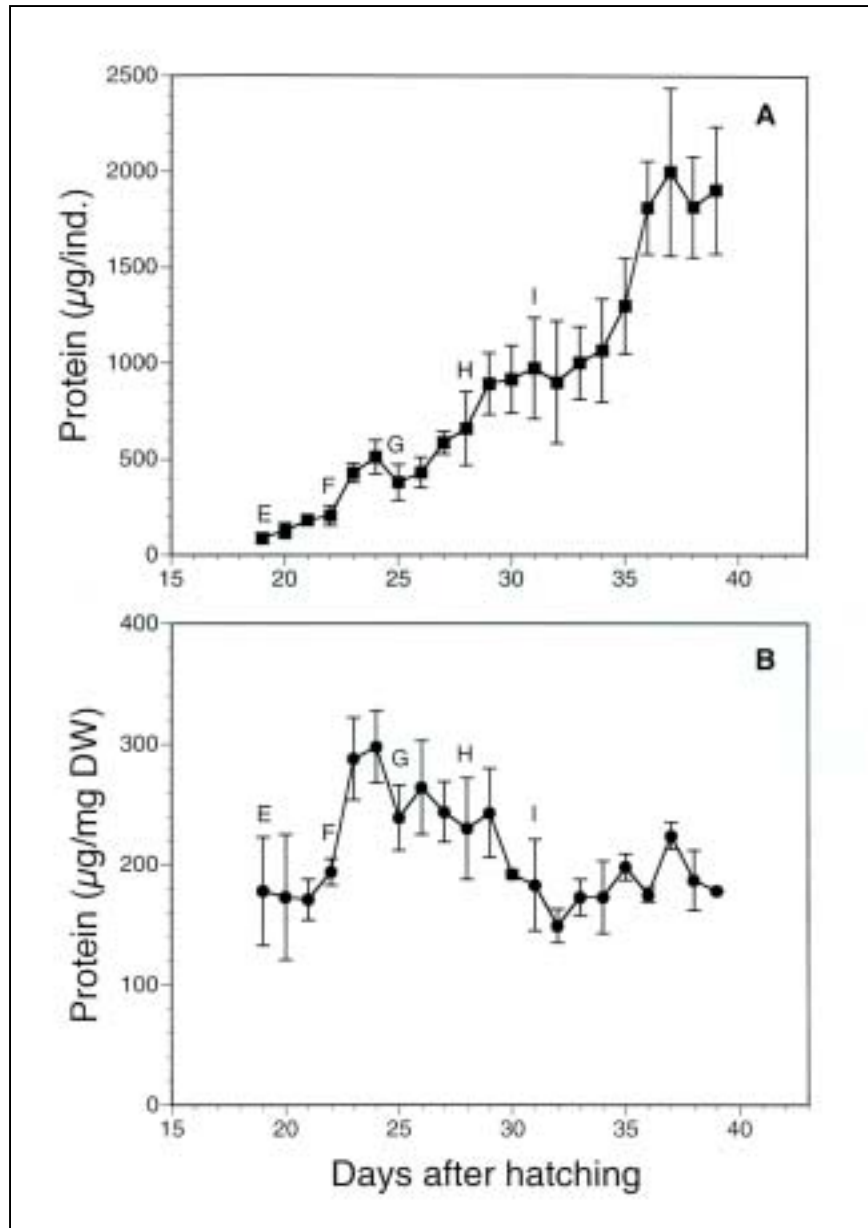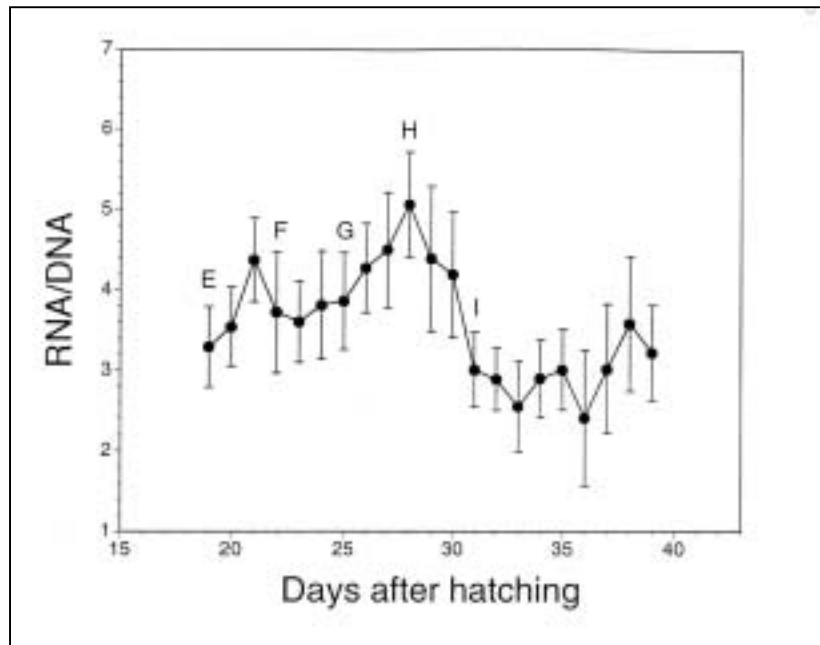

Figure 9. Developmental changes in RNA/DNA ratios of Japanese flounder larvae and juveniles during metamorphosis and early postmetamorphic phase (supplemental 3rd experiment). Values are given as the mean ± SD of five to eight fish. E to I indicate developmental stage.
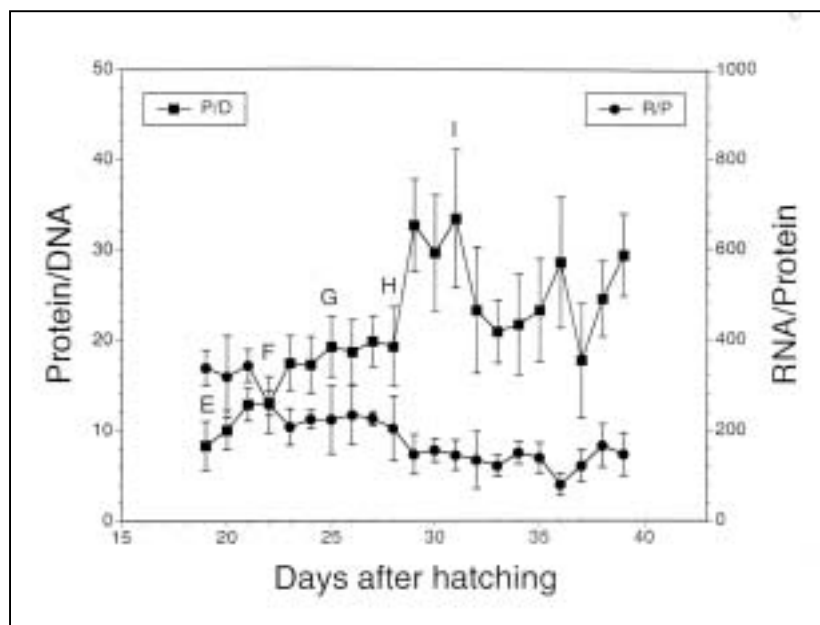
Figure 10. Developmental changes in protein/DNA and RNA/protein ratios of Japanese flounder larvae and juveniles during metamorphosis and early postmetamorphic phase (supplemental 3rd experiment). Values are given as the mean ± SD of five to eight fish. E to I indicate developmental stage.

Table 1 shows the nutritional criteria for evaluating the conditions of wild fish established by RNA/DNA ratios in laboratory rearing experiments. Each were classified either "healthy," if the RNA: DNA was as high as the well-fed hatchery-reared fish, "starving," if the RNA/DNA ranged between that of the well-fed and starved fish during the point-of-no-return (50% mortality), or "dying," if the ratio was below that of the point-of-no-return fish.

The RNA: DNA for wild larvae and juveniles are shown in Fig. 11. Forty-eight pre-metamorphic larvae (B-D stages) were caught in coastal waters from 20 to 113m in depth and 39 late metamorphic larvae and juveniles were caught in the near-shore water ranging from 3.5 to 10m (Table 2). Although individual values varied, the trend indicates a developmental stage-dependent increase in RNA: DNA until H stage, followed by a decrease at I stage. RNA: DNA differed significantly between developmental stages (Fig. 11B, *P*<0.0001). Tukey means comparisons indicated that stage H, with the highest RNA: DNA differed significantly from all other stages, and that stage G also differed significantly from stage I. The ratios of pre-metamorphic phase larvae ranged from 2.7 to 9.4 with an increase in median value at each stage during the two cruises in 1994 (Fig. 11A). Developmental stages differed significantly in their (*P*=0.002). Tukey means comparisons indicated that stage B, with the lowest RNA: DNA, differed significantly from stages E and G, which had the highest ratios, and that stage C also differed significantly from stage E. The general pattern of changes in RNA: DNA for the metamorphic
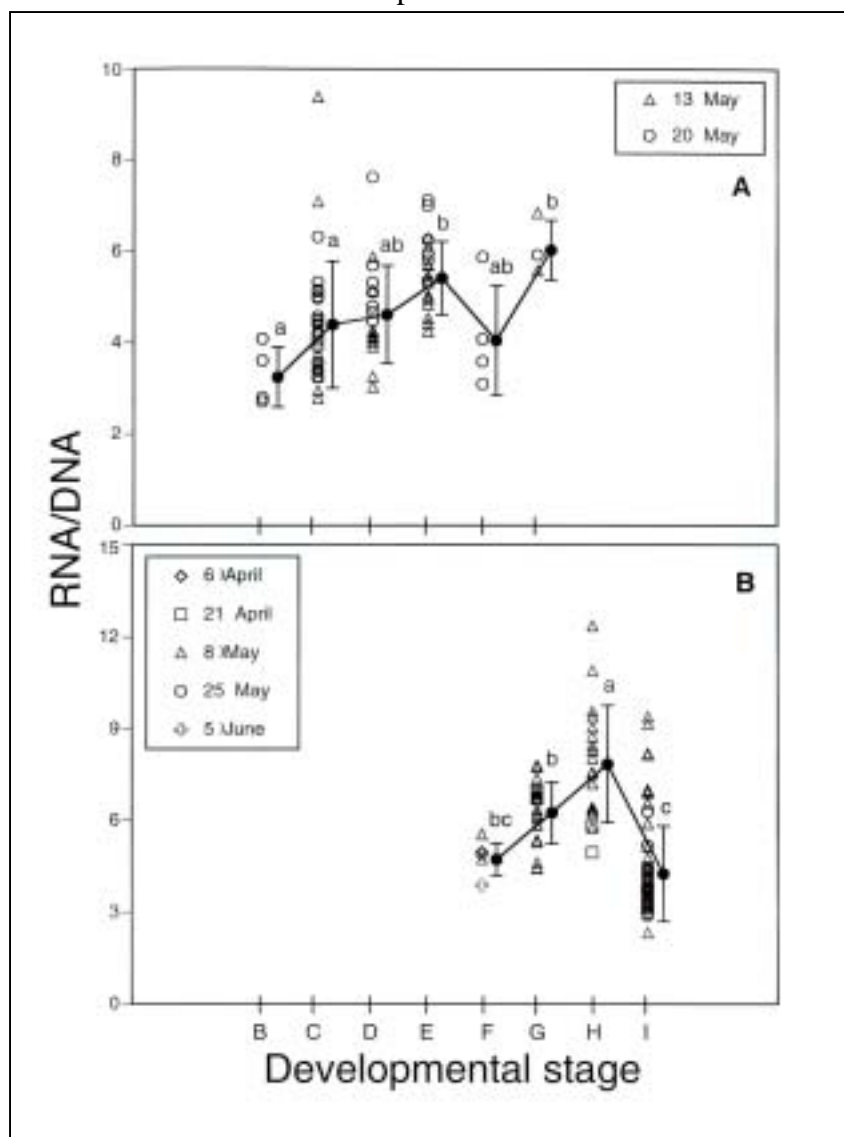
Figure 11. RNA/DNA ratios of wild Japanese flounder larvae and recently settled juveniles collected in the western Wakasa Bay in 1994 (A, N=75) and 1995 (B, N=112). Each closed circle with vertical bar shows the mean value and standard deviation of wild fish at each developmental stage. Vertical bars with same letter are not significantly different (*P*<0.05).

wild larvae was similar to that of the hatchery-reared fish (Fig. 11B). Of the 75 larvae (between C and G stages) collected in 1994, four C and D stage larvae were identified as "starving". Fish collected in 1995 consisted of advanced-stage (G and H stages) larvae and juveniles, but a relatively higher percentage of them considered "starving" (Table 2). The "starving" percentage (54%) at I stage was particularly high. The temperature at each sampling site ranged from 15-16 C in April 1995 to 17-18 C in May and June of 1995 (Table 2).

Discussion

The RNA: DNA of larval Japanese flounder show a drastic increase between 8 (B stage) and 13 DAH following a relatively stagnant state from 4 to 8 DAH (Fig. 8A). Compared with other species, these flounder are characterized by almost no increase for several days after the commencement of feeding, suggesting that a prolonged period of starvation-induced mortality exists. After successfully passing through the critical period at which a shift of energy source occurs, RNA: DNA prominently increases toward day 13, suggesting an occurrence of continuous cell divisions combined with increasing cell size or hypertrophy. Thereafter, the ratio markedly drops until day 17 due to a higher rate of increase of DNA content. This may be caused by greater cell proliferation than protein synthesis. These developmental patterns in RNA: DNA during the pre-metamorphic phase from A to E stages are in agreement with the results of Clemmesen (1987) for herring and turbot larvae, and with the work of Richard *et al.* (1991) on common sole.

Late larval and early juvenile Japanese flounder showed largely fluctuating RNA: DNA during metamorphosis and post-settlement, mainly as a result of fluctuating RNA content versus a relatively gradual increase in DNA content during Exp.2 and 3. In Exp.3, DNA content increased rapidly until 24 DAH, increased slowly at the mid- and late metamorphic stages (G and H) and then rapidly again. This ontogenetic pattern in DNA content appears to be amplified by that of RNA (Fig. 9). It indicates that the early ontogeny of Japanese flounder is composed of cyclic phases of hyperplasia and hypertrophy. Fukuda *et al.*, (1986) reported this kind of cyclic phase in the larval growth of cresthead flounder *Limanda schrenki*. Takii *et al.* (1994) also described a similar result in striped jack *Caranx delicatissimus*. It could therefore be postulated that both RNA and DNA contents are generally more closely linked to developmental stage than to age in larval and juvenile Japanese flounder. Richard *et al.* (1991) made such observations in *Solea solea*. Ehrlich (1974) and Love (1980) also supported the theory that chemical changes are more closely dependent upon larval size than upon age.

A drastic increase in RNA content between the early and late metamorphic stage results in marked increase in protein content and a peak of RNA: DNA. After the peak at the late metamorphic stage (H), the ratio decreased promptly until I_6 stage, primarily due to a drastic increase in DNA versus a decrease in RNA. Similar change was observed by the end of metamorphosis in the plaice larvae (Christensen and Korsgaard, 1999). An increase in RNA: DNA and protein: DNA was repeatedly reflected in the increased dry weight. Greater increasing rates in RNA content during days 19–28 and in DNA content during days 30–39 correspond to hypertrophy and hyperplasia phases of growth in the Japanese flounder. During increases in RNA: DNA it could be speculated that body growth of larvae chiefly occurs by cell enlargement (hypertrophy) resulting from active protein synthesis (Fukuda *et al.*, 1986).

Similar changing patterns in both protein and RNA contents were confirmed during metamorphosis. Protein content leveled off as RNA content decreased between late metamorphic

and post-metamorphic phases. The ontogenetic pattern in protein content during metamorphosis and post-settlement corresponds to those of RNA, indicating that RNA content reflects protein synthesis. A steep increase in both RNA and protein after the post-metamorphic phase was also observed in the DW gain. This fluctuation has led to a marked change in protein: DNA and RNA: DNA around 30 DAH. A decrease in protein: DNA during settlement coincides well with the result of cresthead flounder (Fukuda *et al.*, 1986). Moreover, a marked decrease in RNA: protein between the late and post-metamorphic phases suggests that protein synthesis drop during the non-feeding, settlement period (Tanaka *et al.*, 1996). Thus, energy reserves would be required to complete metamorphosis. These findings indicate that active cell enlargement (hypertrophy) may occur between early and late metamorphic stages, and is followed by higher cell proliferation (hyperplasia) and reduced protein synthesis during the post-metamorphic phase. They also provide evidence for a characteristic cyclic phase of hyperplasia and hypertrophy during metamorphosis. In addition, highly accumulated glycogen in the hepatic tissues was observed during the early metamorphic stage, contrasting to vacuolated hepatocytes found during the post-metamorphic phase (Gwak *et al.,* 1995). It is therefore possible that Japanese flounder larvae save energy by actively synthesizing protein until the metamorphic climax (stage H.) in order to cope with a short-term non-trophic period following settlement.

On the contrary, decreases in RNA: DNA from late metamorphic to post-metamorphic phases could be explained by a higher cell proliferation (hyperplasia) and/or a period of no change in RNA content, resulting from the non-trophic phase. Metamorphic transformation mainly involves tissue degeneration by eventual loss of larval structure, proliferation from larval tissues and organs into those of the adult and the formation of new adult tissues and organs from primordium (Youson, 1988). RNA and DNA dynamics during metamorphosis would correspond well to these degeneration-proliferation developmental events.

RNA content per individual fish dropped drastically throughout the entire starvation phase (Fig. 7), while the DNA content increased or decreased depending upon developmental stage (Fig. 6). Therefore, RNA: DNA dropped due primarily to a much higher reduction rate of RNA content. In fact, starved I stage fish had only 76% of the RNA content of fed fish at I stage. This resulting decrease in the starved larvae and juveniles occurred from the onset of starvation to the day of point-of-no-return. Under deprivation of food supply, a significant amount of RNA could be metabolized at onset, while DNA remained unaffected or slightly increased.

Clemmesen (1987) and Raae *et al.* (1988) made similar observations that DNA content increased with starvation for herring, turbot and cod larvae. Raae *et al.* (1988) also suggested that higher DNA content of starved larvae might be the result of residual cellular energy being used for rapid, unscheduled DNA synthesis, as cellular control mechanisms degenerate due to the lack of sufficient nutrition. Application of RNA: DNA for the assessment of larval nutritional condition has been based on a constant level of DNA under insufficient food supply, as well as significant reduction of RNA. Although there is a large difference between fed and starved fish at 37 DAH with regard to these ratios and total length (Gwak *et al.,* 1999), morphological appearance was similar. Consequently, it is possible that during metamorphosis starved fish mainly utilize their limited energy to maintain metabolism and complete metamorphosis. The increase of DNA content during starvation conditions should be examined in more detail from various aspects, such as species-specific, development-specific, biotic and abiotic factors.

In 1994, all wild larvae were considered healthy except four larvae collected on 13 May, which were determined as starving. In 1995 over 65% starving larvae and juveniles were recognized, all in the H and I stages. Additionally, most were collected in shallower water,

during the late settling season of 25 May and 5 June (Table 2). Since Japanese flounder larvae and juveniles (E-I stages) reared under different temperatures (14 and 22 C) with enough food showed no significant difference in RNA: DNA (Gwak, 1999), when utilizing the established criteria we may negate the temperature effects caused by differences between sampling site and rearing experiment. Tanaka *et al.* (1996) noted a high incidence of empty stomachs in newly settled wild juveniles. This indicate that late larvae and early juveniles caught in shallower water during the late settling season could be more vulnerable to starvation than earlier ones, presumably owing to lower food availability and higher metabolic rate under higher water temperature. Recently settled Japanese flounder prey newly emerged mysid larvae, where abundance in the nursery appears to be variable (Tanaka *et al.*, 1989b). Mysid abundance seems to markedly decrease with the settling season and/or increasing temperature in the Wakasa Bay area (Yamaguchi *et al.,* unpublished data). These field results correspond well to the results obtained in the laboratory where abrupt decreases in RNA: DNA occurred at settlement. They also support the presence of a second critical phase associated with settlement, which can be speculated from ontogenetic changes in the RNA: DNA of hatchery-reared fish.

Changes in feeding and food habits before and after metamorphosis seem to vary with species or type of metamorphosis and/or settlement in flatfishes. Hotta *et al.* (unpublished data) recently demonstrated that a pleuronectid spotted halibut *Verasper variegatus* shows a different type of metamorphosis with continuous feeding during gradual settlement without any sign of feeding cessation. There is no clear reduction of RNA: DNA at settlement. The reduction in Japanese flounder may be more of an ontogenetic nature than environmental effect.

The present study shows that RNA, DNA, and protein content are reliable criterion to investigate the ontogenetic changes during early life history of Japanese flounder. Higher sensitivity of RNA to starvation suggests that RNA: DNA is a good indicator for nutritional condition of larval and juvenile Japanese flounder.

Acknowledgements

Literature Cited

Buckley, L. J. 1979. Relationships between RNA-DNA ratios, prey density, and growth rate in Atlantic cod (*Gardus morhua*) larvae. *J. Fish. Res. Bd Can.* 36:1497-1502.

Buckley, L. J. 1980. Changes in ribonucleic and deoxyribonucleic acid, and protein content during ontogenesis in winter flounder *Pseudopleuronectes americanus*, and effect of Starvation. *Fish. Bull.* 77:703-708.

Bulow, F. J. 1970. RNA-DNA ratios as an indicators of recent growth rates of a fish. *J. Fish. Res. Bd Can.* 27:2343-2349.

Christensen, M. N. and B. Korsgaard. 1999. Protein metabolism, growth and pigmentation patterns during metamorphosis of plaice (*Pleuronectes platessa*) larvae. *J. Exp. Mar. Biol. Ecol.* 237:225-241.

Clemmesen, C. 1987. Laboratory studies on RNA/DNA ratios of starved and fed herring (*Clupea harengus*) and turbot (*Scophthalmus maximus*) larvae. *J. Cons. Int. Explor. Mer.* 43:122-128.

Clemmesen, C. 1988. A RNA and DNA fluorescent technique to evaluate the nutritional condition of individual marine fish larvae. *Meeresforsch* 32:134-143.

Clemmesen, C. 1993. Improvements in the fluorometric determination of the RNA and DNA content of individual marine fish larvae. *Mar. Ecol. Prog. Ser.* 100:177-183.

Ehrlich, K. F. 1974. Chemical changes during growth and starvation of larval *Pleuronectes platessa*. *Mar. Biol*. 24:39-48.

Fogarty, M. J. 1993. Recruitment in randomly varying environments. ICES *J. Mar. Sci.* 50:247-260.

Fukuda, M., Y. Yano, H. Nakano, and M. Sugiyama. 1986. Protein and nucleic acid changes during early developmental stages of cresthead flounder. *Bull. Fac. Fish.* Hokkaido Univ. 167:951-955.

Fukuhara, O. 1986. Morphological and functional development of Japanese flounder in early life stage. *Nippon Suisan Gakkaishi* 55:17-23.

Gwak, W. S., T. Seikai, and M. Tanaka. 1999. Evaluation of starvation status of hatchery-reared Japanese flounder *Paralichthys olivaceus* larvae and juveniles based on morphological and histological characteristics. *Fisheries Sci*. 65:339-346.

Gwak, W. S. 1999. Evaluation of the nutritional status of Japanese flounder *Paralichthys olivaceus* larvae and juveniles, and its application to the wild fish. Ph.D. thesis, Kyoto University, Japan.

Gwak, W.S., et al. 1995. Abst. Metg. Japan. Soc. Fisheries Sci., Sept., 1995, p. 72 (in Japanese).

Hjort, J. 1914**.** Fluctuations in the great fisheries of northern Europe viewed in the light of the biological research. *Rapp. P.-v. Réun. Cons. int. Explor. Mer*. 20:1-228.

Keefe, M. and K. W. Able. 1993. Patterns of metamorphosis in the summer flounder (*Paralichthys dentatus*). *J. Fish Biol*. 42: 713-728.

Love, R. M. 1980. The Chemical Biology of Fishes. Academic Press, New York.

Mathers, E. M., D. F. Houlihan, and L. J. Burren. 1994. RNA/DNA and protein concentrations in fed and starved herring *Clupea harengus* larvae. *Mar. Ecol. Prog. Ser*. 107:223-231.

May, R. C. 1974. Larval mortalities in marine fishes and the critical period concept, pp. 3-19. <u>In</u>: J.H.S. Blaxter (ed.), The Early Life History of Fish. Springer-Verlag, Berlin.

Minami, T. 1982. The early life history of a flounder *Paralichthys olivaceus*. *Nippon Suisan Gakkaishi* 48:1581-1588 (in Japanese).

Phil, L. 1990. Year-class strength regulation in plaice (*Pleuronectes platessa*) on the Swedish west coast. *Hydrobiologia* 195:79-88.

Raae, A. J., I. Opstad, P. Kvenseth, and B. T. Walther. 1988. RNA, DNA and protein during early development in feeding and starved cod (*Gadus morhua* L.) larvae. *Aquaculture* 73:247-259.

Richard, P., J. P. Bergeron, M. Boulhic, R. Galois and J. Person-Le Ruyet. 1991. Effect of starvation on RNA, DNA and protein content of hatchery-reared larvae and juveniles of *Solea solea*. *Mar. Ecol. Prog. Ser*. 72:69-77.

Sale, P. F. 1990. Recruitment of marine species: is the bandwagon rolling in the right direction? *Trends Ecol. Evol*. 5:25-27.

Sato, C., R. Kimura, K. Nakata, S. Umeda, and M. Suzuki. 1995. RNA/DNA ratio of first-feeding larvae of Japanese sardine. *Fisheries Sci*. 61:538-539.

Seikai, T., J. B. Tanangonan, and M. Tanaka. 1986. Temperature influence on larval growth and metamorphosis of the Japanese flounder *Paralichthys olivaceus* in the laboratory. *Nippon Suisan Gakkaishi* 52:977-982.

Takii K, M. Seoka, O. Takaoka, S. I. Furuta, M. Nakamura, and H. Kumai. 1994. Chemical composition, RNA and DNA contents, and alkaline phosphatase activity with growth of Striped Jack larvae through juveniles. *Fisheries Sci*. 60:73-76.

Tanaka, M., T. Goto, M. Tomiyama, H. Sudo, and M. Azuma. 1989a. Immigration, settlement, and mortality of flounder (*Paralichthys olivaceus*) larvae and juveniles in a nursery ground Shijiki Bay, Japan. Neth. *J. Sea Res*. 24:57-67.

Tanaka, M., T. Goto, M. Tomiyama, H. Sudo, and M. Azuma. 1989b. Lunar-phased immigration and settlement of metamorphosing Japanese flounder larvae into the nearshore nursery ground. *Rapp. P.-v. Réun. Cons. int. Explor. Mer.* 191:303-310.

Tanaka, M., M. Kawai, T. Seikai, and J. S. Burke. 1996. Development of the digestive organ system in Japanese flounder in relation to metamorphosis and settlement. *Mar. Fresh. Behav. Physiol.* 28:19-31.

Tanangonan, J. B., H. Nakano, and M. Tanaka. 1998. Changes in DNA, RNA, and protein content during early growth and development of Japanese flounder, *Paralichthys olivaceus*. *Suisanzoshoku* 46:243-252.

Theilacker, G. H. 1978. Effect of starvation on the histological and morphological characteristics of jack mackerel *Trachurus symmetricus* larvae. *Fish. Bull*. 76:403-414.

Theilacker, G. H. and Y. Watanabe. 1989. Midgut cell height defines nutritional status of laboratory raised larval northern anchovy *Engraulis mordax*. *Fish. Bull*. 87:457-469.

Thorisson, K. 1994. Is metamorphosis a critical interval in the early life of marine fishes? *Environ. Biol. Fish*. 40:23-36.

Yin, M. C. and J. H. S. Blaxter. 1986. Morphological changes during growth and starvation of larval cod *Gadus morhua* and flounder *Platichthys flesus. J. Exp. Mar. Biol. Ecol*. 104:215-228.

Youson J. H. 1988. First Metamorphosis, pp.135-196. In: W.S. Hoar and D.J. Randall (eds.), *Fish Physiology*, Vol. 11. Academic Press, New York.

Genetic Diversity Within and Between Hatchery Strains of Japanese Flounder *Paralichthys olivaceus* Assessed by Means of Microsatellite and Mitochondrial DNA Sequencing Analysis[δ]

Masashi Sekino
Tohoku National Fisheries Research Institute
Fisheries Agency
Shiogama, Miyagi 985-0001
JAPAN
Email: sekino@affrc.go.jp

Motoyuki Hara
National Research Institute of Aquaculture
Fisheries Agency
Watarai, Mie 516-0193
JAPAN

Nobuhiko Taniguchi
Graduate School of Agricultural Science
Tohoku University
Sendai, Miyagi 981-8555
JAPAN

Abstract

We assessed genetic divergence within and between hatchery and wild populations of Japanese flounder *Paralichthys olivaceus* by means of microsatellite and mitochondrial DNA (mtDNA) sequencing analysis. Three hundred individuals derived from three hatchery strains and 190 individuals from three wild populations were examined. All 11 microsatellites screened were polymorphic in all samples. Sequences of the mtDNA control region of Japanese flounder were highly variable; of approximately 443 base pairs sequenced, 132 sites were variable among 490 individuals. The number of microsatellite alleles and mtDNA haplotypes, and mtDNA haplotype diversity showed marked reductions in the hatchery strains compared with the wild populations. Both molecular markers yielded high values of *F*-statistics between the hatchery strains, and between the hatchery strains and wild populations. According to a phylogenetic tree topology on the basis of inter-individual genetic relatedness as estimated from microsatellite data, the three hatchery strains were genetically separated, possibly caused by random genetic drift. The DNA markers employed in this study should provide an ideal means for genetic monitoring of Japanese flounder hatchery stocks.

Introduction

The Japanese flounder *Paralichthys olivaceus* is a flatfish species widely distributed throughout coastal areas of Japan and forms an important fishery resource. Recent interest has been directed toward stocking of hatchery -reared fish into natural sea areas to increase the exploitable resource mass. We are concerned with the potential genetic impact of the stocking practice on the wild fish stocks since loss of genetic variability in most hatchery stocks is typical,

---

[δ] *§ An earlier version of this paper is available in Aquaculture 2002 213: 101-122.*

and this may possibly result in the loss of disease resistance or in the reduction of population's capability to adapt to new environments (Allendorf and Phelps, 1980).

Here, we present an application of two kinds of molecular marker to identical sets of Japanese flounder samples derived from three hatchery stations and three natural sea areas in order to evaluate genetic condition of hatchery strains comparing with that of wild populations. The molecular markers used in this study were eleven microsatellite loci (Sekino and Hara, 2000) and a section of mitochondrial DNA (mtDNA). We also aimed to document potential uses of the molecular markers for further genetic monitoring of Japanese flounder hatchery stocks.

Materials and Methods

Fish Samples

Wild Japanese flounder samples were collected from the Japan Sea in Hokkaido Prefecture (HKD, 50 individuals), from Tottori Prefecture (TTR, 69 individuals) and from the Pacific Ocean in Chiba Prefecture (CHB, 72 individuals). Hatchery fish were provided from a hatchery station in Hokkaido Prefecture (HH, 100 individuals), in Tottori Prefecture (HT, 100 individuals) and in Miyagi Prefecture (HM, 100 individuals). Figure 1 shows the geographical positions of the samples examined. All individuals in the HH strain were F_1 offspring from approximately 110 wild flounder (50 females and 60 males) sampled from off Hokkaido Prefecture.

The HT strain was founded using approximately 300 individuals. One hundred individuals (50 females and 50 males) were mated in each of three aquarium tanks and the offspring sampled from each tank were communally reared in one tank. The candidate parents were both wild flounder sampled from Tottori Prefecture and brood-stock hatchery-reared potentially over several

Figure 1. Geographic positions of six Japanese flounder samples. The black dots indicate three sample sites of wild populations; the open dots indicate the location of hatchery stations

generations. There are no available records showing the number of siblings used for this strain. The HM population originated from approximately 60 wild flounder (30 females and 30 males) sampled from Miyagi Prefecture, comprised of F_1 offspring of the wild captives. Genetic information on the candidate parents from the three hatchery strains was not available.

Polymorphism Screening

Eleven microsatellites, *Po1*, *Po13*, *Po25A*, *Po26*, *Po33*, *Po35*, *Po42*, *Po48*, *Po52*, *Po56*, and *Po91* were screened in this study. The PCR amplification condition for each locus is available in Sekino and Hara (2000). Mendelian inheritability for each locus was verified in our previous study (Sekino and Hara, 2001). Microsatellite polymorphisms were screened using an ALF express automated DNA sequencer (Amersham Pharmacia Biotech, Uppsala, Sweden).

According to a complete nucleotide sequence of Japanese flounder mtDNA genome (Saitoh *et al.,* 2000, GenBank accession AB028664) we designed one set of PCR primer pair to amplify approximately 480 base pair (bp) segments flanking the tRNA^{Pro} gene and the left domain of the control region: 2 primers were placed in the tRNA^{Thr} gene (forward primer: 5'-GTT AGA GCG CCA GTC TTG TA-3') and the middle of the control region (reverse primer: 5'-CCT GAA GTA GGA ACC AAA TGC-3'). The PCR amplification was carried out in a 10 µl reaction mixture, which included 10 pmols of each primer, 100 µM of dNTPs, 10 mM Tris-HMl (pH8.3), 50 mM KCl, 1.5 mM MgCl$_2$, 0.2 units of DNA polymerase (ExTaq^{TM}, Takara, Shiga, Japan). Approximately 50 ng of template DNA. PCR cycles were as follows: 3 min at 95, 30 cycles of 15 s at 95 , 30 s at 57 , and 30 s at 72 , and final elongation for 5 min at 72 . Sequencing analysis for the PCR amplification products was performed using an ABI 373A stretch DNA sequencer (Applied Biosystems, Foster City, CA USA). Sequences were determined from both directions.

Statistical Analysis

Microsatellite allele frequencies and expected heterozygosity (*He*) of each population at each locus were estimated using an ARLEQUIN version 1.1 software package (Schneider *et al.,* 1997). The observed heterozygosity (*Ho*) was calculated directly from the observed genotypes. We used the ARLEQUIN program to estimate an overall inbreeding coefficient (F_{IS} ; Weir and Cockerham, 1984).

Sequence alignment of mtDNA sequence data was performed using a sequence editor DNASIS software package (HITACHI, Tokyo, Japan). The number of variable sites, haplotype frequency distributions and haplotype diversity were calculated using the ARLEQUIN program. The haplotype diversity was based on the formula $h = (1 - \Sigma x_i^2)\, n / (n -1)$, where x_i is the frequency of a haplotype and *n* is the sample size (Nei and Tajima, 1981).

Overall *F*-statistics (Weir and Cockerham, 1984) was estimated based on both microsatellites (F_{ST}) and mtDNA sequences (Φ_{ST}) using the ARLEQUIN program. Genetic relationships between individuals within and between hatchery strains were estimated. First, we defined the term "allele frequency in an individual" as follows: if individual *X* had genotype *AA* at locus *L*, the frequency of allele *A* at that locus in the individual *X* was defined as *A* =1.0; if individual *X* had genotype *AB* at that locus, the frequency of allele *A* and *B* in the individual *X* was defined as *A* = 0.5 and *B* = 0.5. Then, we estimated inter-individual genetic similarity according to a formula $I = \Sigma X_i Y_i / (\Sigma X_i^2 \Sigma Y_i^2)^{1/2}$, where X_i and Y_i is the frequency of *i* th allele for each locus in the individual *X* and *Y*, respectively. We calculated the *I* values for all possible pairwise combinations of individuals for all loci, and then a pairwise genetic distance measure was calculated as $D = (1 - I_k)$, where I_k is the average of the *I* values calculated for each locus. A phylogenetic tree topology based on the distance measure was constructed according to a neighbor-joining method (Saitou and Nei, 1987). This analysis was performed using 80 individuals per hatchery strain, that is, a total of 240 individuals were used for this analysis.

Results

Table 1 summarizes the microsatellite variabilities. The variability estimated for the hatchery strains is characterized as substantial reductions of the number of alleles per locus (hatchery strains*:* 5.9-10.7; wild populations: 15.3-18.2). Overall expected heterozygosity (*He*) ranged from 0.59 to 0.71 in the 3 hatchery strains, and from 0.75 to 0.76 in the 3 wild populations.

Table 1. Microsatellite variabilities in six Japanese flounder samples.

	Hatchery strains			**Wild populations**		
	HH	**HT**	**HM**	**HKD**	**TTR**	**CHB**
Number of loci examined	11	11	11	11	11	11
Sample size	100	100	100	50	69	72
Hardy-Weinberg disequilibrium [1]	8	4	3	0	0	0
Number of alleles per locus (*A*)	10.7	5.9	10.0	15.3	17.5	18.2
Overall observed heterozygosity (*Ho*)	0.72	0.57	0.72	0.77	0.78	0.75
Overall expected heterozygosity (*He*)	0.71	0.59	0.70	0.75	0.76	0.75
Overall F_{IS}	-0.021	0.018	-0.037	-0.023	-0.028	-0.001
P [2]	0.86	0.20	0.98	0.88	0.95	0.55

[1] *Number of loci that showed significant departure from Hardy-Weinberg's equilibrium. The probability was tested analogously to Fisher's exact test in the Markov-chain method, with initial K of sequential Bonferroni correction (Rice, 1989) K=11 (P<0.005)*

[2] *Probability value associated with the F_{IS}.*

Sequences containing the tRNA[Pro] gene (71bp) and the left domain of the mtDNA control region turned out to be highly variable: of approximately 443 nucleotides, which we unambiguously determined for a total of 490 individuals, there were 132 variable sites consisting of 149 base-substitutions with 5 single base pair insertion/deletion (Fig. 2). Accordingly, a total of 179 haplotypes were identified in the 490 individuals. Haplotypic variabilities estimated for the 6 samples are summarized in Table 2. The 3 hatchery strains did not share any common haplotypes with each other. All hatchery strains had a lower haplotype diversity (*h*=0.692-0.798) than the wild populations (*h*=0.998). There were marked reductions as regards both the number of mtDNA haplotypes and haplotype diversity even in the first-generation hatchery strains (i.e., the HH and HM strains).

```
|  tRNAᴾʳᵒ  →                    .           .                    .                    |
TCAGAAAAAGGAGATTTCAACTCCTACCCCTAACTCCCAAAGCTAGGATTCTAGCGTTAAACTATTTTCTG

|  Control region →

 .   .  .  . .  ..            .. .         .         .    . .  . .      ..
GGAACATATGTTTTATGAAAATTAATATACATATATGTAATTACACCATATATTTATAGTAAACATTAAGTCCGATGTACAA
 .. .......... .   ..... .....  .....     .. .       ..: .......:...     .   .
GACACAAATGGATGTGAACAAAACATGGTGTCAAACATTCATATACCAGCTATATAACTAAATATGTACAAAAACCAA
 .............  ..  ............  .....   ..
ACCTATAAGGTATACGATAAAGAATTGAAGACTAATCGAAACTTTACACCGAACACAACCTTCATATGTCAAGTTATAC
         ..  ...........  .          .   .  .
CAAGACTCAAACCTCTGTCGATCCCAAA−TTCCCATGCAGTAAGAGCCTACCATCAGTTGATTACTTAATGCCAACGGT
      .  .. .          .. :      .
TAT TGAAGGTGAGGG ACAAAA AT TGTG GG GGT TTCACACAGTGAAC TAT TCC TG
```

Figure 2. Sequence of the tRNAPro genes and the left domain of the control region of mtDNA of Japanese flounder. Dots indicate variable sites found in at least one haplotype; dashes indicate the single nucleotide deletion/insertion.

Table 2. Mitochondrial DNA variabilities in 6 Japanese flounder samples.

| | Hatchery strains | | | Wild populations | | |
	HH	HT	HM	HKD	TTR	CHB
Sample size	100	100	100	50	69	71
Number of variable sites	43	29	37	76	87	103
Number of haplotypes	14	4	7	48	65	66
Haplotype diversity *(h)*	0.798	0.692	0.793	0.998	0.998	0.998

Table 3 shows overall *F*-statistics estimated based on both microsatellites (F_{ST}) and mtDNA sequences (Φ_{ST}). A high level of sample-differentiation with statistically significant F_{ST} (Φ_{ST}) was estimated among the hatchery strains (*P*<0.001). We compared each hatchery strain with the geographically proximal wild population (i.e., HH *vs* HKD, HT *vs* TTR, and HM *vs* CHB). The F_{ST} and Φ_{ST} values estimated for all of the 3 combinations were significantly different from zero (*P*<0.001).

Table 3. Estimates of F_{ST} (Φ_{ST}) value based on microsatellites and mtDNA sequences.

Sample Combinations	Microsatellites		MtDNA	
	F_{ST}	P^{*1}	Φ_{ST}	P
Among hatchery strains	0.088**	0.000	0.187**	0.000
Among wild populations	0.004*	0.003	0.005	0.134
HH *vs.* HKD	0.026**	0.000	0.084**	0.000
HT *vs.* TTR	0.086**	0.000	0.150**	0.000
HM *vs.* CHB	0.034**	0.000	0.079**	0.000

*[*1] Probability value associated with the F_{ST} (Φ_{ST}) is shown. The F_{ST} (Φ_{ST}) values significantly greater than zero, based on random allelic permutation testing, are noted by adding *=P<0.005 and **=P<0.001.*

According to the NJ tree topology constructed on the basis of the inter-individual genetic similarity (Fig. 3), almost all of individuals derived from each hatchery strain were closely combined, excepting 3 instances as 3 individuals derived from the HH strain were closely clustered with individuals from the HM strain.

Figure 3. NJ-tree topology, as determined by midpoint rooting, that shows the genetic relationships among 240 individuals randomly chosen from three hatchery strains. Genetic similarity between individuals was calculated on the basis of 11 microsatellite genotypes by using a formula analogous to the genetic identity index between populations (Nei, 1987).
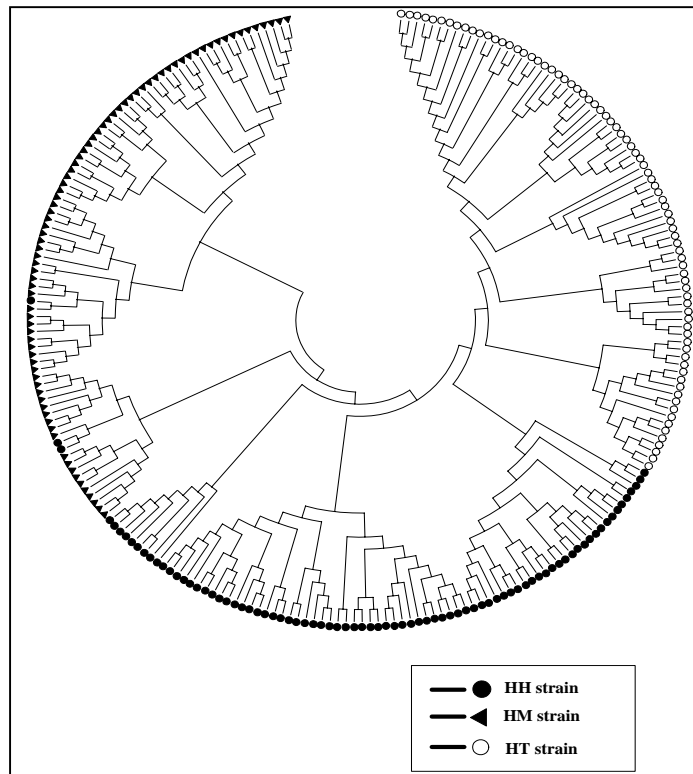
Discussion

A substantial reduction of the number of alleles per locus observed in all of the 3 hatchery strains suggests that each hatchery strain was bottlenecked (Table 1). This is due most likely to the small a number of effective parents when each population was founded. Overall expected heterozygosity (*He*), however, did not show pronounced differences between the hatchery strains and wild populations excepting 1 instance: a significant reduction of the *He* value was observed in the HT strain (see below). These results are not surprising since an estimate of heterozygosity could be inflated if a hatchery strain was founded using heterozygous parents. We therefore consider that the *He* value should not necessarily be useful to evaluate a potential reduction of genetic variation so far as in a first-generation hatchery strain. As an effect of bottlenecking and inbreeding increases, a possibility of significant losses of heterozygous individuals however should increase. It is plausible to consider that the significant reductions of *He* value detected in the HT strain would be caused by population bottleneck together with occurrences of inbreeding events when this strain was founded. This is because the HT strain was founded using both wild caught flounder and brood-stock maintained in this hatchery station, the level of inbreeding however seems not to be high since homozygote excess was not evident in this strain (*Ho*/*He*=0.97), and since the F_{IS} value estimated for this strain was indeed higher compared with other samples but not significant (F_{IS}=0.018, *P*=0.20).

Small a number of mtDNA haplotypes identified in the hatchery strains (4-14 haplotypes) was in contrast to large a number of haplotypes identified in the wild populations (48-66 haplotypes) (Table 2). Considering the fact that the large number of haplotypes were observed in the wild populations (160 haplotypes in 190 individuals), and that the HH and HM strains were first-generation of wild caught flounder, it seems reasonable to assume that the number of haplotypes detected in the HH strain (14 haplotypes) and the HM strain (7 haplotypes) represents the actual number of female parents in each strain. Given that the HH strain was founded using approximately 50 females and the HM strains using 30 females, it can be concluded that only 25% of the candidate female parents for both strains (HH strain: 14/50; HM strain: 7/30) were effective to found each strain.

High F_{ST} and Φ_{ST} values estimated for between the hatchery strains, and between the hatchery strains and wild populations (Table 3), do indicate that there is pronounced genetic differentiation between these samples, possibly caused by random genetic drift. The NJ tree topology showing inter-individual genetic relationships seems to be consistent with genetic drift occurred in hatchery strains as well.

The present study demonstrated that the simultaneous use of the 11 microsatellite loci and the sequences of the mtDNA control region is a powerful approach to monitor genetic condition in Japanese flounder hatchery strains. It should be noted that further extensive stocking practice without any consideration of genetic impact on wild populations might possibly result in irredeemable losses of alleles/haplotypes in natural stocks. The only way to minimize the genetic impacts is to improve the genetic management for all hatchery strains by means of monitoring the genetic variability, estimating precise effective population size. A parentage analysis should provide the most efficient means for this purpose, and we suggest that both microsatellite and mtDNA sequencing technique have the potential to be of great use for this approach.

Acknowledgments

Literature Cited

Allendorf, F. W. and S. R. Phelps. 1980. Loss of genetic variation in a hatchery stock of cutthroat trout. *Trans. Am. Fish. Soc.* 109:537-543.

Crow, J. F. and M. Kimura. 1965. Evolution in sexual and asexual populations. *Am. Nat.* 99:439-450.

Nei, M. and F. Tajima. 1981. DNA polymorphism detectable by restriction endonucleases. *Genetics* 97:145-163.

Rice, R. W. 1989. Analyzing tables of statistical tests. *Evolution* 43:223-225.

Saitoh, K., K. Hayashizaki, Y. Yokoyama, T. AsahidaT., Toyohara, and Y. Yamashita., 2000. Complete nucleotide sequence of Japanese flounder (*Paralichthys olivaceus*) mitochondrial genome: structural properties and cue for resolving teleostean relationships. *J. Heredity* 91:271-278.

Schneider, S., J. M. Kueffer, D. Roessli, and L. Excoffier. 1997. ARLEQUIN (version 1.1) -A software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Sekino, M. and M. Hara. 2000. Isolation and characterization of microsatellite DNA loci in Japanese flounder *Paralichthys olivaceus* (Pleuronectiformes, Pleuronectoidei, Paralichthydae). *Mol. Ecol.* 9:2200-2202.

Sekino, M. and M. Hara. 2001. Inheritance characteristics of microsatellite DNA loci in experimental families of Japanese flounder *Paralichthys olivaceus. Mar. Biotech.* 3:310-315.

Weir, B. S. and C. C. Cockerham. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution* 38:1358-1370.

Tracking Released Japanese Flounder *Paralichthys olivaceus* by Mitochondrial DNA Sequence

Tetsuo Fujii
Japan Sea National Fisheries Research Institute
Fisheries Research Agency
1-5939-22, Suido-cho, Niigata 951-8121
JAPAN
Email: tefujii@affrc.go.jp

Abstract

A method of tracking released Japanese flounder *Paralichthys olivaceus* was developed by using the extremely high sequence variability of the mitochondrial DNA, particularly in the control region. The determination of the mother of a released flounder is possible by sequencing the mitochondrial DNA control region because of its variability and maternal inheritance. In this method, sequences of hatchery-reared juveniles should be analyzed first. The sequence data are then registered in a database, so that when the flounder is later caught its origin can be determined. We can obtain information about the genetic variability of hatchery-reared stocks in the sea, as well as the migration of the released flounder. Although other methods have been used to track released flounder, mitochondrial DNA sequence appears to be less harmful for juveniles and potentially less expensive.

The Japanese flounder *Paralichthys olivaceus* is one of the most important species for the coastal fisheries in Japan. More than 30 million hatchery-reared juveniles have been released annually for the enhancement of stocks. It is well known that the wild flounder migrate widely as they grow (Minami, 1997). To elucidate the migration of the released flounder it is necessary to carefully manage the genetics as well as evaluate stocking effectiveness. Previous tagging studies have been performed using external tags or chemical markings. External tags may be harmful to juveniles (most juveniles are released less than 10 cm in total length) and difficult to place on large numbers of fish. On the other hand, chemical markings, such as fluorescent marking in otoliths, are comparably easy to apply, but the patterns of the marks are limited. To avoid these tagging obstacles, a method of tracking released Japanese flounder by mitochondrial DNA (mtDNA) sequence was developed. In this paper, the concept and advantages of the mtDNA tag are described. Potentials for use of the mtDNA tag and future programs are also introduced briefly.

Concept of mtDNA Tag

In this method, sequences of hatchery-produced juveniles must first be analyzed and registered in a database. The mtDNA of wild Japanese flounder is characterized by the extremely high sequence variability especially in the control region (D-loop region). The direct sequencing of the 350 base pairs (bp) in the first half of the control region showed that 126 sites (36.0%) were variable. The sequence differences between individuals were up to 8.3%, with an average

of 4.3%, as 54 haplotypes were detected from 55 individuals (Fujii and Nishida, 1997). This means that almost all flounder have their own unique sequences and mothers of hatchery-reared juveniles can be determined because of its high variability and maternal inheritance. In Japan there is at least one hatchery in every prefecture, each having its own broodstock. This makes hatchery-rearing of juveniles localized. Since most of the hatcheries are supported by the prefectures, most juveniles are released in their home prefectures. When the released flounder are recaptured later, their origins of rearing and release can be determined by their mtDNA sequences.

Fortunately, most of hatchery-reared juveniles have abnormal pigmentation, called melanism, on their blind sides. Therefore it is easy to distinguish released flounder from wild ones. DNA analysis is also possible from a single fish scale preserved in 99.5% ethanol. All that would be necessary at the fish market is to pick one scale from each flounder having melanism on the blind side.

Advantages of the mtDNA Tag

The mitochondrial DNA tag has the following advantages:

- The DNA tag will never change or detach.

- DNA analysis is possible from a single fish scale, which is less harmful to the fish and reduces the cost of purchasing them to check for tags.

- It is possible to obtain data about individual fish.

- The cost for the DNA tag does not depend on the number of fish released, but rather on the number of haplotypes present in hatchery-reared juveniles and the number of recaptured fish. It costs approximately $2.50 per sample of muscular tissue and $3.25 for scales, including reagents, tubes and tips, and maintenance fee for gears.

- One sample may be analyzed in less than one day, and up to 200 samples may be analyzed per week.

- It is possible to get data about genetic diversity of both hatchery-reared juveniles and recaptured fish as a haplotypic diversity of mtDNA.

In conclusion, the mtDNA tag method is immutable, easy to sample, easy to analyze, economical and multipurpose.

Potential of mtDNA Tag

The efficiency of the mtDNA tag relies on high sequence variability and distinctive appearance between wild and released fish. The mtDNA sequence of the red sea bream *Pagrus major*, one of the most important species used in Japanese stock enhancement programs, is highly variable (Tabata and Mizuta, 1997) and easily distinguished from wild fish by the lack of an inter-nostril epidermis (Yamazaki, 1998). The mtDNA tag is therefore considered a useful tool for tracking hatchery-reared red sea bream. Differences in appearance between wild and hatchery-reared fish enable scientists to analyze more fish and determine their origins.

Future Programs

      We have just begun the project to elucidate the migration of released Japanese flounder along the Sea of Japan under the cooperation of prefectural staffs. Preliminary results showed that released Japanese flounder began to migrate in their second winter mainly against the Tsushima current, which flows from the southwest to northeast.

      Some of them migrated more than 300 km and flounder from several hatcheries were recaptured together in many regions. The haplotypic diversity of a group of recaptured fish is usually higher than those released from a single hatchery, due to a mixture of flounder released from several hatcheries and their conserved genetic diversity until recaptured. The mitochondrial DNA tag is useful not only for the elucidation of the migration but also for the monitoring of genetic diversity. It is expected that these methods will be put into effect in the near future.

Literature Cited

Fujii, T. and M. Nishida. 1997. High sequence variability in the mitochondrial DNA control region of the Japanese flounder *Paralichthys olivaceus*. *Fish. Sci*. 63:906-910.

Minami, T. 1997. Life History, Biology and Stock Enhancement of Japanese Flounder Koseisha Koseikaku, Tokyo, pp 9-24 (In Japanese).

Tabata, K. and A. Mizuta. 1997. RFLP analysis of the mtDNA D-loop region in red sea bream *Pagrus major* population from four locations of western Japan. *Fish. Sci*. 63:211-217.

Yamazaki, A. 1997. Deformities of the pectoral fin and inter-nostril epidermis and the discrimination of the artificially produced and wild red sea bream *Pagrus major* in release-recapture research. *Saibai Giken* 26:61-65 (In Japanese).

Preliminary Aspects of Genetic Management for Pacific Threadfin *Polydactylus sexfilis* Stock Enhancement Research in Hawaii

Michael D. Tringali
Florida Marine Research Institute
100 Eighth Avenue South East
St. Petersburg, Florida 33701
USA
Email: mike.tringali@fwc.state.fl.us

David A. Ziemann
The Oceanic Institute
41-202 Kalanianaole Highway
Waimanalo, Hawaii 96795
USA

Kenneth C. Stuck
Gulf Coast Research Laboratory
University of Southern Mississippi
Ocean Springs, Mississippi 39564
USA

Abstract

Preliminary aspects of genetic management for Pacific threadfin stock enhancement research at the Oceanic Institute (OI) have been focused on genetic stock identification and broodstock management. To investigate genetic structure in threadfin populations potentially impacted by stock enhancement, wild specimens from 4 locations in Hawaii ($n = 41$) and from 3 locations in Oahu ($n = 32$) were assayed by sequencing 1045 base pairs of the mitochondrial DNA (mtDNA) control region. Due to the large number of nucleotide sites assayed, haplotype diversity was high (99.3%); a total of 61 unique haplotypes were observed from the 73 individuals assayed. However, nucleotide diversity was low (0.64%). No phylogeographic structure was evident in clustered haplotypes. Genetic variance was partitioned predominantly among individuals within populations (98%); approximately 1% of the genetic variance occurred between the threadfin from the islands of Oahu and Hawaii. Haplotype distributions did not differ significantly among these two locations. These data, which are preliminary, are suggestive of high gene flow on a regional basis. The female effective population size, estimated using a Maximum Likelihood Metropolis-Hastings sampling method, ranged approximately 200,000-400,000. The sampled population appears to have undergone a large, historical expansion. Taken together, data are consistent with an evolutionarily recent colonization of the species in the Hawaiian Islands. Preliminary studies for broodstock management are focusing on levels of relatedness among female broodstock and female contributions to OI progeny groups. Using mtDNA sequencing, maternity surveys were performed for cultured progeny groups which contained both normal individuals and individuals exhibiting a particular morpho-anatomical deformity. The condition appeared to be manifested ubiquitously and randomly among progeny of the contributing females. Data provided no evidence that inbreeding or maternal effects are causative factors. If controlled by a single autosomal (dominant or recessive) gene, our preliminary results suggest that the condition has very low penetrance/expressivity in the wild threadfin population.

Introduction

The Pacific threadfin, *Polydactylus sexfilis* (Valenciennes *in* Cuvier and Valenciennes, 1831), is widely distributed throughout the Indo-Pacific region. Because of recent morphological studies, the taxonomy of *Polydactylus* is currently in flux (*see* Mishra and Krishnan, 1993; Fricke, 1999; Motomura *et al.*, 2000; Motomura *et al.*, 2001). Fricke (1999) suggested that *P. sexfilis and P. sextarius* should be synonymized. Motomura *et al.* (2001) disagreed, offering a revised morphological description of *P. sexfilis* based on comparisons of type material and new specimens collected range-wide to specimens (including relevant type material) from the putative congeneric species *P. kuru*, *P. nigripinnus*, *P. plebeius*, and *P. sextarius*. Interestingly, in Motomura's *et al.*, (2001) study, type material for *P. kuru* was indistinguishable from specimens of *P. sexfilis* and thus the authors regarded *P. kuru* to be a junior synonym of the latter. The western margin of the Pacific Plate represents a biogeographic boundary for the majority of *Polydactylus* species (Springer, 1982). Of approximately 20 threadfin species in the Indo-Pacific, the Pacific threadfin is one of two or three species that have colonized the interior portion of the plate. It characteristically occurs near oceanic islands and is considered to be less dependent upon large river systems than its regional congeners.

In the Hawaiian Islands, Pacific threadfin is a popular sport fish that also supports localized commercial fisheries. Unfortunately, annual catches of threadfin in some areas have declined dramatically over the last few decades and have not recovered despite implementation of stringent fishery regulations. Beginning in 1993, the Oceanic Institute undertook an experimental program to determine if cultured Pacific threadfin can be used to replenish depleted Hawaiian fisheries (reviewed in Ziemann, 2002, this issue). Between 1993-1998, cultured threadfin were tagged and released along the windward coast of Oahu in order to study factors critical to survival and recruitment (e.g., the effects of various stocking densities, size-at-release, and release habitat; Friedlander and Ziemann, in press; Ziemann and Friedlander, in press). Early results were promising – cultured threadfin comprised approximately 10% threadfin in Oahu's recreational catch in the years following stocking. As part of the Oceanic Institute's effort to implement a responsible program of stock enhancement, current research involves the genetic study of native and cultured Pacific threadfin.

Among the cultured threadfin recaptured in coastal waters were several large, fully-mature females. Where there exists an opportunity for reproductive exchange between cultured and wild organisms, there also exists the potential for genetic impact. Tringali and Leber (1999) outlined three types of genetic hazards that should be considered when cultured and wild stocks are mixed: Type I -- among-stock (introgressive) transfer of deleterious genes; Type II -- pre-release genetic modification of cultured organisms (including domestication, purging of diversity, and inbreeding); and Type III -- genetic swamping of recipient stocks from overwhelming contributions of cultured organisms. To manage these genetic risks, a baseline genetic survey of the potentially impacted wild population is necessary. This survey should include the following components: 1) quantification of levels of genetic diversity in the population, 2) geographic/spatial partitioning of genetic variation, 3) quantification of temporal genetic variance (drift) and estimation of effective population sizes, and 4) characterization of the population's demographic history.

The mitochondrial genome offers a ready source of genetic characters for many of the above-listed analyses. Mitochondrial DNA is relatively easy to obtain and assay (Palumbi, 1996); there is no need for expensive and time-consuming marker development and testing.

MtDNA is haploid, typically maternally inherited, and not subject to recombination. MtDNA generally evolves in a "clocklike" fashion in percoids, although relative rates of mtDNA sequence evolution may vary among congeneric species (e.g., Tringali *et al.*, 1999). Because rates of nucleotide substitution in mitochondrial genes of vertebrates are 5-10 times higher than in most nuclear genes (Wilson *et al.*, 1985; Billington and Hebert, 1991), resolution in population-level mtDNA studies is generally good. In particular, examination of nucleotide variation in the non-coding "control region" of mtDNA has proven to be a powerful tool for studying genetic structure in marine species (e.g., Stepien, 1995; Graves, 1998; Reeb, 2000). Moreover, this marker has been used to delineate interbreeding wild fish stocks prior to enhancement (e.g., Seyoum *et al.*, 2000; Garber, 2001), to compare levels of genetic diversity between hatchery and wild stocks (e.g., Sekino *et al.*, 2002, *this issue*), to determine effective sizes for hatchery cohorts (M. Tringali, unpublished data), to identify a significant maternal component to red drum hatchling survivorship during "common-garden" artificial rearing (M. Tringali, unpublished data), and to track hatchery organisms after release (e.g., Fujii, 2002, this issue).

Here, we report the preliminary results of a survey of mtDNA control region sequence for wild Pacific threadfin from two regions within the Hawaiian Island chain. Sequence data were also obtained for members of a threadfin progeny group produced at the Oceanic Institute to investigate if there was a relationship between maternity and a recurrent anatomical defect in hatchlings. Data collection and laboratory analyses are ongoing. The resultant information will be useful in the development of a risk-adverse plan of genetic management for the Pacific threadfin stock enhancement program.

Material and Methods

Sample Collection and Laboratory Analyses

Locations of capture and sample sizes for wild specimens are given in Fig. 1. In addition, a total of 49 threadfin, reared from captive broodstock at OI, were assayed. These represented a subsample of progeny that had been pooled at hatching from two OI spawning groups. Twenty-four of the cultured specimens exhibited a particular developmental defect – i.e., a missing or deformed opercle. The remaining 25 specimens appeared to be developmentally normal.

Total genomic DNA was obtained from each specimen by using an organic extraction procedure based on that of Taggart *et al.* (1992). The resulting DNA pellets were resuspended in 50 µL of TE buffer (10 mM Tris, 1 mM EDTA; pH 8.0) and quantified using fluorescence spectrophotometry, as described by Gallagher (1994). DNA concentrations were then adjusted to 100 ng/µL using 1 mM Tris and stored at -20 C. Species-specific primers designated *ThreadPro* (5'-CCTACTGCTTCAAAGAAGAG-3') and *ThreadPhe* (5'- TTGTGCTCACAGGGGTTGTC-3') were designed (K. Stuck and W. Grater, unpublished) and used to amplify the entire mtDNA control region. PCR amplifications were conducted in replicate 50 µL reactions containing 200 ng template DNA, 1.5 mM $MgCl_2$, 200 µM deoxynucleotide triphosphates (dNTP), 0.3 µM of each primer, and 3.0 units of *Taq* DNA polymerase with 10X PCR buffer (Amersham Life Science).
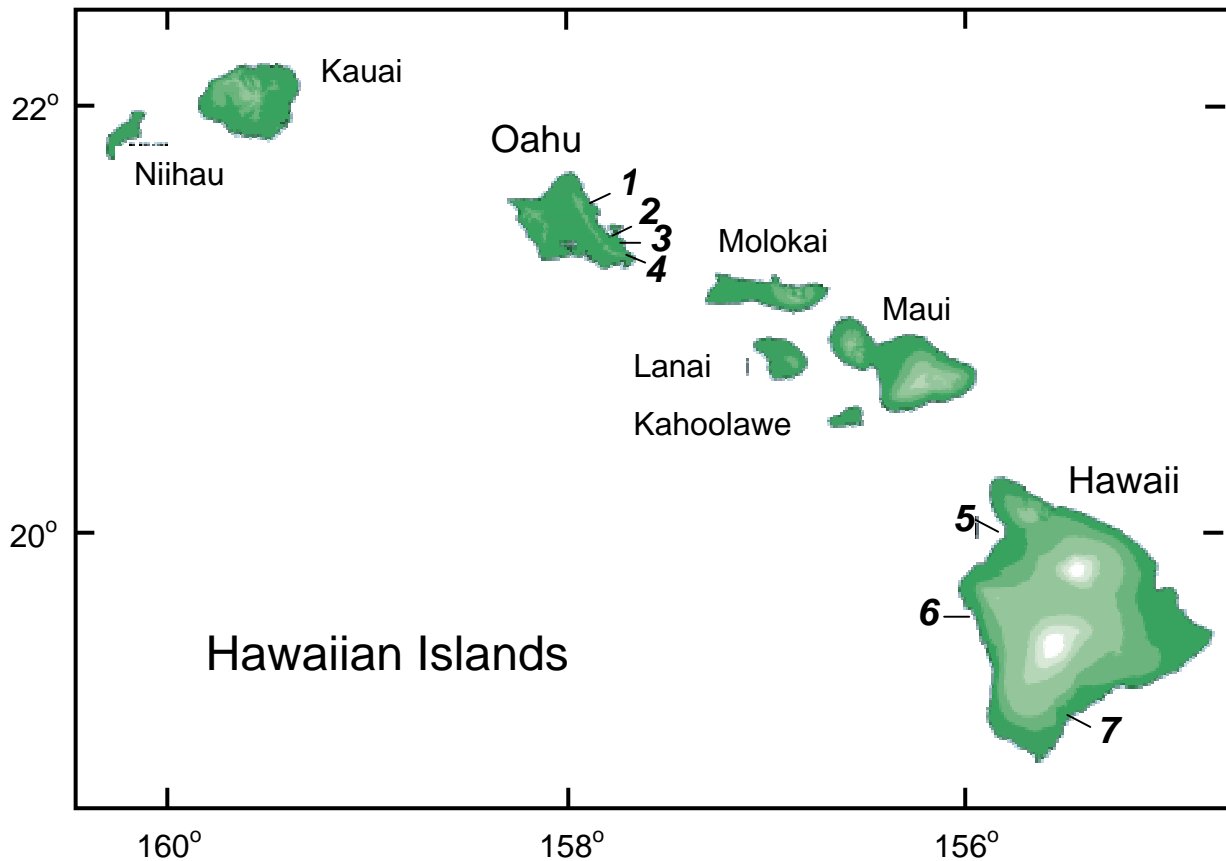
Figure 1. Capture location of Pacific threadfin specimens assayed in this study. For Oahu and Hawaii, regional sample locations and sample sizes are as follows: (1) Kaaawa, *n*=4;   (2) Kailuana, *n*=17;   (3) Kalama, *n*=5;   (4) Waimanalo, *n*=6;   (5) Hapuna, *n*=16;   (6) Kialua, *n*=18; (7) Punaluu, *n*=7.

All PCR products were visualized on 1% TBE-agarose gels containing ethidium bromide (0.5 µg/mL). DNA bands were excised from the gels and purified using the QIAquick Gel Extraction Kit. Purified amplicons were ligated into vector DNA and used to transform competent JM109 cells which, in turn, were cultured on Luria-Bertani (LB)/ampicillin plates containing x-gal and IPTG. Blue/white selection was used to identify colonies potentially containing inserts. Plasmid DNA was isolated from minipreps using Wizard® *Plus* DNA Purification System (Promega, Inc.) and the presence of inserts was confirmed by digestion with EcoRI. Plasmid DNA was then purified using the PEG method described by Nicoletti and Condorelli (1993) and sequenced using a Licor 4200 automated sequencer. Sequence data were aligned with CLUSTALW (Higgins and Sharp, 1988).

Genetic Analyses

Levels of mtDNA control-region variability within samples of wild Pacific threadfin were examined by computing the nucleotide (Tamura and Nei, 1993) and haplotype (Nei, 1987)

diversity indices using the Arlequin 2.0b2 software package (Schneider *et al.*, 1999). Nei and Li's (1979) nucleotide divergence (*D*) values were estimated within and between groups. Statistical testing for population structuring involved 1) an *r* x *c* exact test (Raymond and Rousset, 1995) of a contingency table based on haplotype frequencies and 2) a hierarchical analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992). Significance levels of all test statistics were evaluated by randomization testing as described in Arlequin. The level of migration (gene flow) between the islands was estimated from the fixation index (F_{ST}; Slatkin, 1991). Genealogical relationships between mtDNA control region haplotypes were reconstructed as follows: MEGA (Version 2.1, Kumar *et al.*, 2001) was used to generate pairwise matrices of corrected sequence divergence values (Tamura and Nei, 1993); the pairwise-deletion option for indels was invoked. From these, a neighbor-joining tree (Saitou and Nei, 1987) was constructed; statistical support for each node was obtained using the bootstrap procedure in MEGA.

To estimate female effective population size ($N_{e,f}$) for wild Pacific threadfin in Hawaii, the Metropolis-Hastings sampling method of Kuhner *et al.*, (1995), as implemented in FLUCTUATE (Version 1.3, mkkuhner@genetics.washington.edu), was applied to the entire set of mtDNA control-region sequences for wild specimens. Fu's (1997) F_S statistic and Tajima's (1989) *D* statistic were estimated using Arlequin. A mismatch distribution was constructed and analyzed for evidence of recent population expansion or bottleneck. Harpending's (1994) raggedness index (*r*) was computed for each distribution. Demographic parameters (and their associated SSD *P* values) were estimated using a non-linear least squares approach (Schneider and Excoffier, 1999).

To test the hypothesis that the condition of opercular deformity in cultured threadfin hatchlings was associated with maternal relatedness, all pairs of assayed individuals were classified as follows: both individuals were deformed/related maternally; both deformed/ unrelated maternally; both normal/ related maternally; both normal/ unrelated maternally. The proportion of deformed individuals that shared a dam and the proportion of normal individuals that shared a dam were each compared to the null proportion (50%) using *V* tests (DeSalle *et al.*, 1987).

Results and Discussion

Intraspecific Diversity Levels in Wild Pacific Threadfin

Our data indicate that the mitochondrial DNA control region represents a good source of intraspecific genetic characters for threadfin studies. For the preliminary survey, 1,045 base pairs within the control region were routinely and consistently scored. From these, 105 sites were polymorphic, including 3 sites that contained indels. The transition/transversion ratio was 4:1. A 10-base-pair sliding window (Fig. 2) shows the distribution of nucleotide substitutions over the DNA region assayed. Although substitutions occurred over the entire length of the sequenced segment, a conserved block exists in the center of the segment from which internal (nested) PCR primers could be developed if needed. There were no hypervariable areas within the threadfin control region. This represents a fortunate circumstance from the standpoint that mutational "hotspots" may confound the analytical models employed herein due to backmutations, among-site rate heterogeneity, and other violations of model assumptions. Additionally, it suggests that the control region should be a source of useful phylogenetic/taxonomic characters for morphologically similar *Polydactylus* (*see* Introduction).
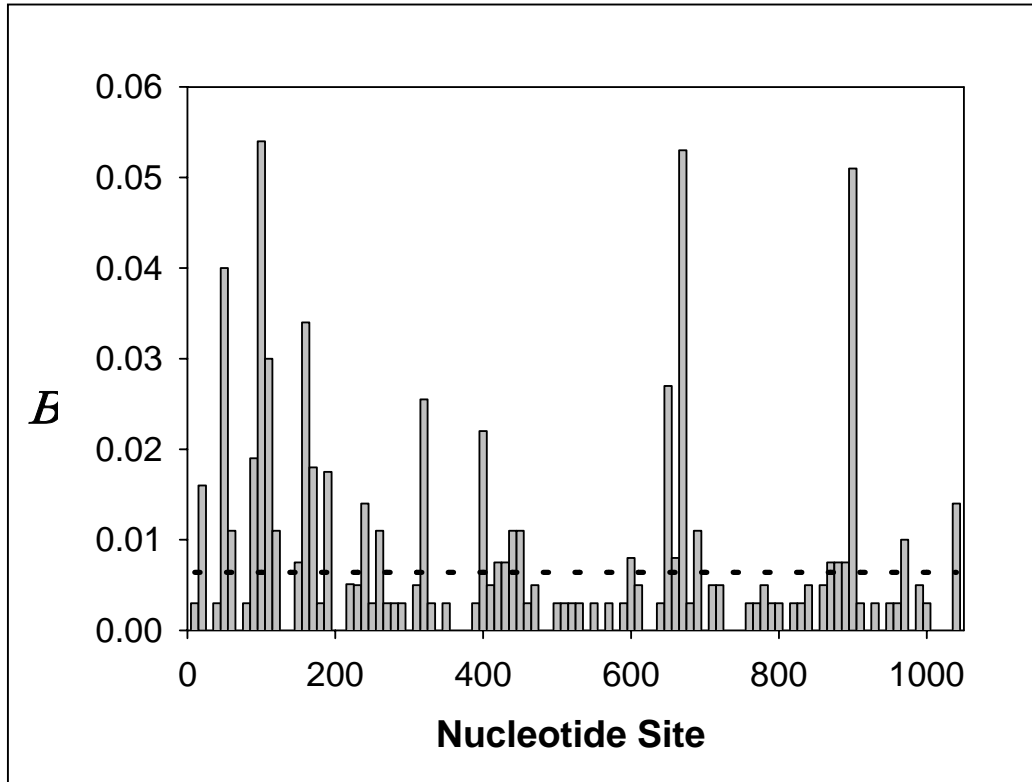
Figure 2. Ten base-pair sliding window of mitochondrial DNA control region variability for all wild Pacific threadfin. The dashed line depicts the mean value of nucleotide diversity ($Б$=0.0064).

Among the wild specimens, 61 unique haplotypes were observed. Haplotype diversity (the probability of randomly choosing two individuals bearing different haplotypes) was high (h=99.3%). However, for wild Pacific threadfin, nucleotide diversity (the average number of pairwise differences among haplotypes per site) was somewhat low (π=0.0064), especially in comparison to that commonly observed in control regions of other marine percoids (e.g., *Sciaenops ocellatus* – 0.030 [Seyoum *et al.*, 2000]; *Lutjanus campechanus* – 0.025 [Garber, 2001]; *Cynoscion nebulosus* – 0.024 and *Archosargus probatocephalus* – 0.039 [S. Seyoum, M. Tringali and T. Bert, unpublished data]). However, the value was in keeping with levels of control region nucleotide diversity reported for three of four species of Hawaiian amphidromous fishes (range 0.004 to 0.010; Chubb *et al.*, 1998). The greatest genetic distance between any one pair of Pacific threadfin haplotypes was 0.018. Potential reasons for the low levels of "per site" mtDNA variation in threadfin are discussed below. Despite these low levels, sufficient marker variation existed for analyses of genetic structure, gene flow, and historical demography because of the large number of polymorphic sites assayed.

<u>Genetic Structure and Gene Flow</u>
The first step in effective fisheries management is to identify the "unit stock." The dynamics of organisms within this management unit will be driven by the same natural and

anthropogenic processes and respond similarly to regulation; however, the response of organisms in one unit stock will be independent of (although not necessarily different from) the response in another. Similarly, the first step in a program of genetic management is to identify the "genetic" stock(s) – i.e., the discrete gene pool(s) into which cultured organisms will reproductively integrate. Geographic delineation of genetic stocks structure in Hawaiian Pacific threadfin will permit informed management of Type I genetic impacts and establish hierarchical levels at which Type III impacts should be considered.

For statistical analysis of threadfin genetic structure, samples collected from locations within the islands of Hawaii and Oahu, respectively, were pooled *a priori*; the "Hawaii" group ($n=41$) was compared to the "Oahu" group ($n=32$). Sizes of samples within these groups are as yet too small to make valid intra-group comparisons. Average numbers of pairwise nucleotide differences for the Hawaii and Oahu groups were 7.646 and 5.312, respectively. The average pairwise difference between these two groups was 6.590. Because the between-group value was approximately the same as within-group values, a hypothesis of divergence between the groups was unsupported. Likewise, the result of the exact test for the geographic distribution of haplotypes was consistent with a hypothesis of panmixia ($P=1.000$ ₄ 0.000). The majority of individuals ($n=54$) had unique haplotypes. Thirty-four unique haplotypes were present among individuals in the Hawaii group; 20 were present among individuals in the Oahu group. The distribution of the 7 haplotypes that were shared within and between groups is given in Table 1.

Table 1. Distribution of shared mtDNA control region haplotypes within and between samples of Pacific threadfin, *Polydactylus sexfilis*, from the Hawaii and Oahu groups. See Fig. 1 for the composition of samples for the two groups. Fifty-four haplotypes were present in single individuals and are not listed here.

Haplotype Designation	Hawaii Group	Oahu Group
PT-006	2	2
PT-017	2	0
PT-022	1	1
PT-030	1	1
PT-039	1	2
PT-042	0	2
PT-045	0	4

There was no evidence of between-group geographic isolation in the population genealogy reconstructed using the neighbor-joining algorithm (Fig. 3). The genealogy was essentially star-like; shallow lineages were predominant. All bootstrap values on tree nodes (not shown) were less than 95% and typically less than 50%. The tree topology was geographically diffuse; neighboring haplotypes were often collected from different islands.
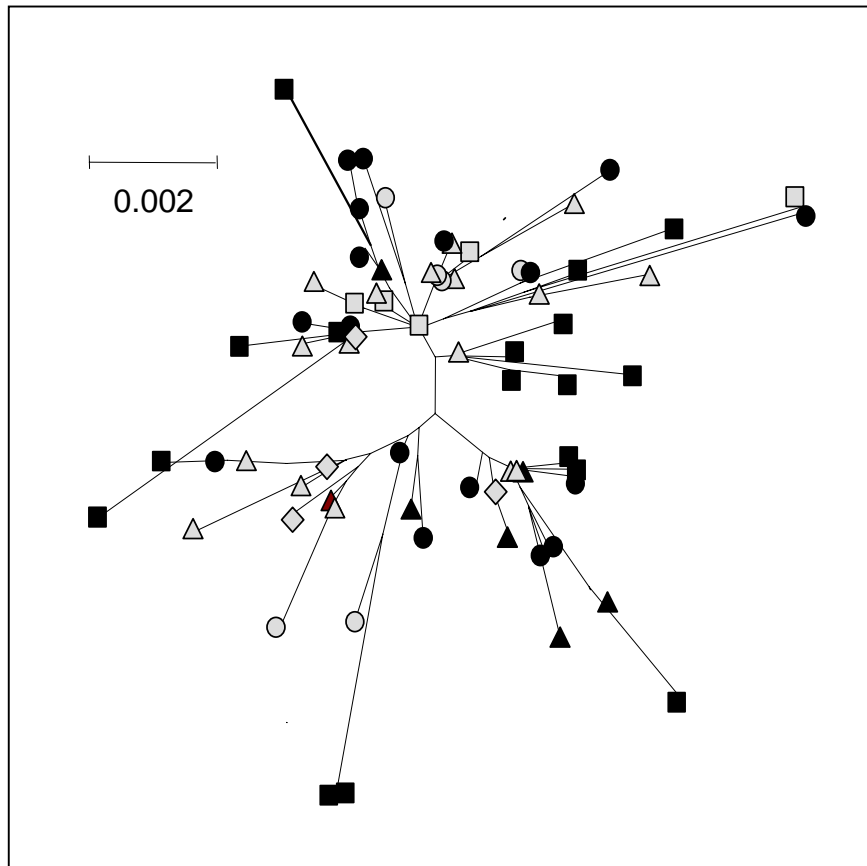
Figure 3. Neighbor-joining cluster of mitochondrial DNA control region haplotypes for all wild Pacific threadfin based on Tamura-Nei (1993) pairwise distances. Geographic locations from which haplotypes were obtained are depicted as follows: Oahu; Kalama, *grey squares*, Waimanalo, *grey circles*, Kailuana, *grey triangles*, Kaaawa, *grey diamonds*. Hawaii, , Kialua, *black squares,* Hapuna black circles, *Punaluu,* black triangles.

AMOVA was used to partition molecular variance within and between the Hawaii and Oahu groups (Table 2). Approximately 1.5% of the overall variance was due to differences between the two groups. Statistical significance of the observed between-group variance was tested by permuting haplotypes among groups; there was a 6.5% (i.e., reasonable) probability of obtaining a greater or equivalent between-group variance by chance alone. Ultimately, larger sample sizes for locations within islands will permit computation of within-island variance components for Hawaii and Oahu threadfin, respectively. If the hypothesis of panmixia is valid, these variance components would also be expected to be on order of 1-2%. The fixation index was low ($F_{ST} = 0.0153$, $P = 0.108$), suggesting that gene flow is high between these two groups and thus high for Pacific threadfin on a regional basis.

Table 2. Analysis of molecular variance (AMOVA, Excoffier *et al.*, 1992) for mitochondrial DNA control region haplotypes of Pacific threadfin from Hawaii.

Source of Variation	df	Sum of Squares	Variance Component	Percentage of Variation
Among Groups	1	5.165	0.05152	1.53[*]
Within Groups	71	235.266	3.31361	98.47

[*] *The probability that a larger value would have occurred by chance was 0.06549.*

To investigate the effects that reducing the length of the assayed marker would have on analytical results, the above analyses were repeated using only the first 500 nucleotides. In this case, the average numbers of pairwise nucleotide differences within the Hawaii and Oahu groups were 4.534 and 3.168, respectively and the average pairwise difference between these two groups was 3.908. Compared to the above divergence values, these values were reduced approximately by a factor of 2, as expected due to the reduction in the number of sites examined. But again, a hypothesis of divergence between the two groups was not supported. The test result for the geographic distribution of haplotypes was again consistent with a hypothesis of panmixia (P=1.000 $\pm$ 0.000). In the AMOVA, 1.3% of the overall variance was due to differences between the two groups. There was a slight loss of statistical power in this AMOVA; the probability of obtaining a greater or equivalent between-group variance component by chance alone was 9.7%. The fixation index remained low (F_{ST} = 0.0129, P=0.090). In general, these results show that the length of the marker could be reduced as described without significant loss of resolution for extended studies of gene flow and genetic structure in threadfin. Additional resolution would more likely be obtained by the inclusion of more specimens in each of the existing samples. The shortened segment would be easily obtainable using *ThreadPro* and an internal primer. Shortening the length of the marker via an internal primer would reduce laboratory effort and expense since sequence could be obtained directly from amplicons. The model used herein to estimate female effective population size (*see below*) is sensitive to the number of characters (nucleotides) employed and the analysis benefited from the large size of the segment.

Overall, the preliminary results indicate that the mitochondrial genomes of Pacific threadfin from the islands of Hawaii and Oahu are not distinct. This circumstance can arise from two scenarios. First, it is possible that female reproductive exchange has occurred with sufficient frequency over time to homogenize or prevent divergence in threadfin mitochondrial genomes within the sampled range. If so, then the genetic sampling in this study has occurred within the geographic boundaries of a single genetic stock. Alternatively, it is possible that, if Oahu and Hawaii threadfin are indeed reproductively isolated from one another, that insufficient evolutionary time has passed since separation for significant genomic-level mtDNA divergence. Larger sample sizes obtained from the current island locations will be helpful in discriminating between these scenarios. For now, absent evidence of genetic stock structure in Hawaiian Pacific

threadfin, all wild specimens were pooled for analyses of female effective population size and demographic history.

Female Effective Population Size

The "effective population size" (N_e) is an important parameter for population genetic surveys (see Tringali and Bert, 1998). This parameter is related to the rate at which genetic change (e.g., inbreeding, drift, allele fixation or loss) is expected to occur in a population. In this study, the mtDNA sequences from the 73 wild individuals were used to obtain an estimate of the female effective size (N_{ef}) of the sampled Pacific threadfin genetic population. The method described in Kuhner *et al.* (1995) was employed. Briefly, the distribution of time intervals between coalescent events in mtDNA geneologies inferred from a population sample is shaped by the parameter 1. This parameter is related to the female effective population size and the mutation rate (:) of the sequenced DNA segment (i.e, for a haploid gene, $1 = 2\ N_{ef}$:). A Markov-chain sampling of the genealogies was used to compute the maximum likelihood estimate of $1 = 0.655$, which applies to the time of population sampling. The corresponding estimate of N_{ef} is relatively insensitive to the mutation rate (per site) of the sequenced marker. Adopting a very low divergence rate of 2% per million years, : equates to 8.5×10^{-7} (assumes a generation time of 6 years for threadfin). Adopting the highest recorded divergence rate for the control region in vertebrates (32% per million years; Merilä *et al.* 1997), $: = 1.6 \times 10^{-6}$. Thus, it is likely that the female effective size of the Pacific threadfin population at present is between approximately 205,000 and 385,000 individuals. We note that the range of values given here does not represent a confidence interval; rather, it reflects uncertainty in the mutation rate for threadfin. The approach of Kuhner *et al.* (1995) accounts for changing population sizes. The maximum likelihood estimate of the population growth rate parameter, *g*, was 933.367 for threadfin. Positive values of *g* are indicative of net population growth (expansion) over time.

A large female effective size for Pacific threadfin in Hawaii would be a positive sign for the present genetic health of the population. Presumably the overall effective size (for both sexes) is correspondingly high. Maintenance of population fitness hinges on a population's ability to remove maladapted or lethal genes through the process of natural selection. Once introduced, the probability that a particular deleterious gene will persist or become fixed in a population is principally a function of its particular selective effect, the initial frequency at which it enters the population, and effective population sizes during the course of its segregation. In general, unless swamped by a multitude of related individuals having maladapted genomes, populations having large N_es will incorporate the majority of selectively beneficial genes and remove the majority of those that are deleterious. In contrast, populations having small N_es, unable to overcome the stochastic effects of drift, may suffer reduced levels of population fitness from an increased mutation load and/or disruption of coadapted genomes. The expectation from the above results is that the present mutation load in Hawaiian Pacific threadfin is low.

Demographic History of Pacific Threadfin in the Hawaiian Islands

Over the long-term, the loss of adaptive variation and the accumulation of deleterious mutations represent potential hazards to population viability. Significant risk from these hazards may exist in populations having undergone significant periods of low N_e, even if current N_es are high. The evolutionary age of the population is also a factor – older populations are expected to have a larger mutation load than younger populations of similar effective size due to the accumulation of slightly deleterious alleles. Sudden occurrences of extreme inbreeding, as may

result from Types II and/or III stocking impacts, may be more problematic for populations having a large mutation load. Therefore, the demographic histories of populations significantly influence their long- and short-term susceptibilities to naturally or artificially induced genetic change. Fortunately, past demographic fluctuations (i.e., population expansions and contractions) often leave genetic signatures encoded in DNA sequence (reviewed in Harpending *et al.*, 1998).

For threadfin, the estimate of Fu's (1997) statistic was a very large, negative value (F_S = -73.295), which may indicate that there was a recent expansion in the sampled population. Figure 4 depicts values of Tajima's (1989) *D* over the assayed region of mtDNA. The overall estimate of Tajima's statistic (D = -2.33, P = 0.00193) was significantly different than the value expected under the neutral mutation hypothesis. This finding is consistent with hypotheses of marker non-neutrality or a recent founding/bottleneck event. Results from other studies of control region variation in (drift/mutation) equilibrium populations, including marine percoid shorefishes (e.g., *Sciaenops ocellatus*, *Cynoscion nebulosus*, and *Archosargus probatocephalus*; S. Seyoum and M. Tringali, unpublished data) are consistent with neutral mutation. Thus, unless there was a recent "sweep" of control-region variation (see Ballard and Kreitman, 1995) via hitchhiking, demographic explanations most likely apply to threadfin.
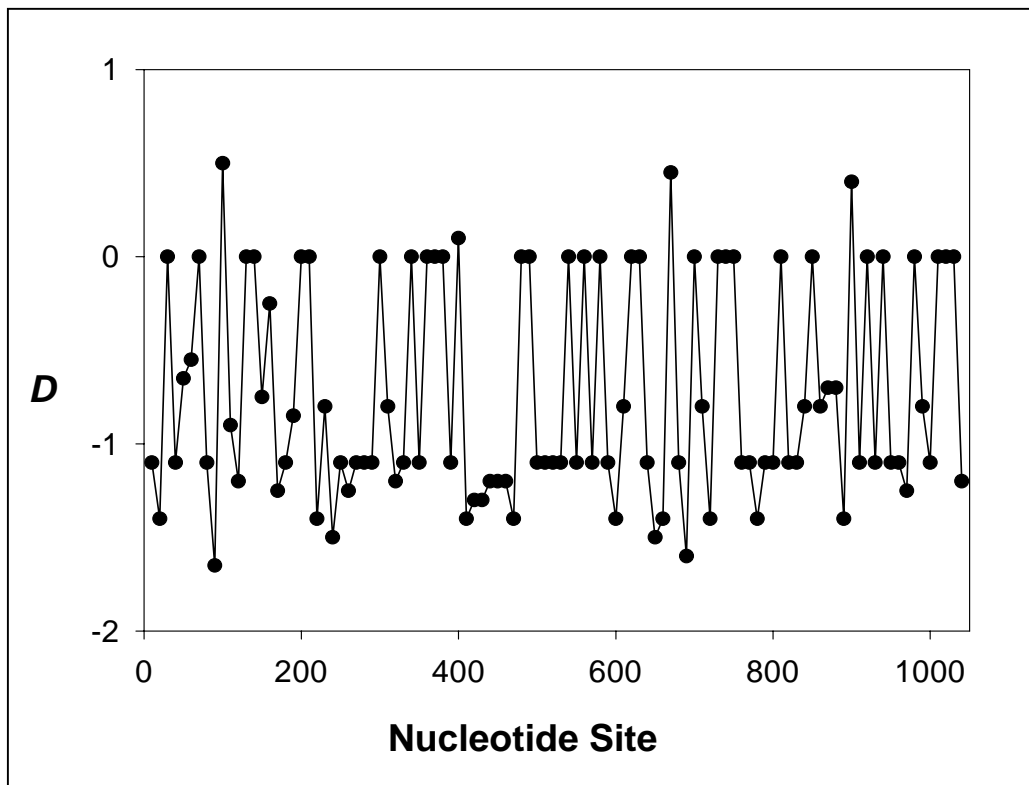
Figure 4. Ten base-pair sliding window showing values of Tajima's (1989) *D* for the assayed region of mtDNA control region in wild Pacific threadfin.

Figure 5 shows the observed and expected distributions of pairwise sequence differences (*i*) among threadfin specimens. The mean and variance of the observed distribution was 6.612 and 9.836, respectively. The observed data exhibited a good fit to that predicted by the "sudden expansion" model of Rogers (1995). The sum of square deviations between the observed and expected distributions (*SSD* = 0.0008) was not significantly different (*P* = 0.66) from a simulated distribution that assumes that the estimated parameters ϑ, I_0, and I_1 are the true ones (*see* Schneider and Excoffier, 1999). The mean square error (MSE) measuring the fit of the data to the sudden expansion model was 0.001131 whereas the MSE measuring its fit to an "equilibrium" (no growth) model was 0.050125 (see Merilä *et al.*, 1997). Harpending's raggedness index was very low (*r* = 0.007), indicating that the observed distribution was unimodal. Thus, as in the analyses of *g*, F_S, and *D*, the analysis of the mismatch distribution was consistent with a hypothesis that the sampled Pacific threadfin population recently underwent an expansion.
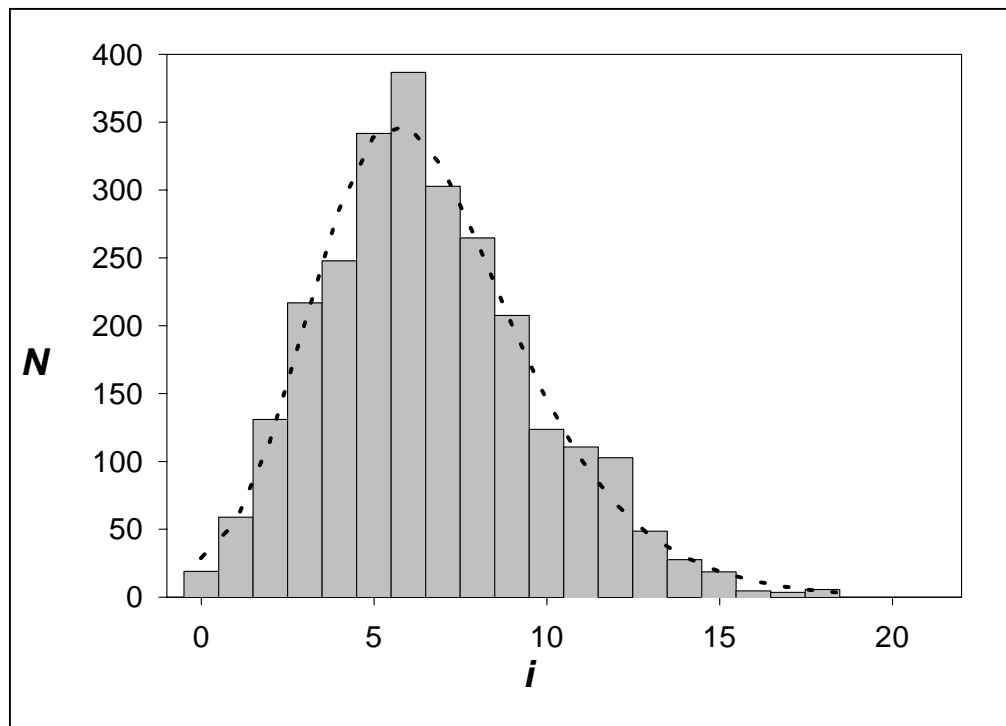
Figure 5. Distribution of the pairwise number of nucleotide differences among *N* pairs of individuals for wild Pacific threadfin. Empirical distribution represented by vertical bars. The expected distribution as predicted by the "sudden expansion" model of Rogers (1995) is depicted by the dashed line.

The demographic parameter ϑ represents a mutational time scale such that the number of generations that have elapsed since a population was founded is approximately ϑ divided by two times the overall (not per site) mutation rate (Rogers and Harpending, 1992). For the sampled Pacific threadfin population, ϑ was estimated to be 5.004. Applying the broad range of control-region divergence rates described in the above section, the estimated time of founding for the

sampled population was approximately 14,000 to 28,000 years ago. This range of founding times is interestingly low considering the presumptive geological ages of the Hawaiian Islands (from approximately 1 to 5 million years old for the southernmost to northernmost islands) and could be explained by at least three scenarios. First, Pacific threadfin may be a nascent species, in which case, the genealogy and diversity-level observed herein would be representative of samples assayed from throughout the entire Indo-Pacific range. This scenario would be testable via the addition of specimens from a distant location (e.g. Japan, Marshall Islands, Caroline Islands). Second, the sampled Hawaiian population (or, less likely, the entire species) could have undergone a severe bottleneck or selective sweep just prior to the estimated founding time. The estimates of I_0 and I_1 suggest that the bottleneck size would have been less than approximately 1000 females and the subsequent expansion size approximately two orders of magnitude greater than 1000 (which is consistent with the above estimate of $N_{e,f}$). However, the leading edge of the mismatch distribution is not consistent with a bottleneck scenario (Rogers and Harpending, 1992). That is, the leading edge of distributions in bottlenecked populations based on 1000 control region nucleotides would characteristically be expected to be ragged and, because of stochastic retention of older lineages, contain many peaks at values of $i > 25$. In threadfin, there are no peaks beyond $i = 18$ and the distribution is smooth. Third, the Hawaiian population could have been colonized (founded) recently by migrants from another Indo-Pacific population. This scenario is not unreasonable given Hawaii's well-known biogeographic history of fish fauna. It is also testable via comparison to a distant sample.

Etiology of Opercular Deformity in Pacific Threadfin

During captive propagation, OI threadfin hatchlings occasionally exhibit a developmental defect resulting in a partially or fully missing gill plate. Osteological and morpho-anatomical defects in cultured fishes can be caused by various factors, both genetic and nongenetic. Examples of commonly reported nongenetic factors include water quality, nutrition, water temperature (particularly during larval rearing), and phenotypic maternal effects. Known genetic causes or factors include inbreeding depression, autosomal single locus variants, and various types of genotypic maternal effects. Often, multiple, interacting causes exist for a given condition. Opercular complex deformity (OCD) manifests in a variety of freshwater and marine cultured species, including cyprinids (Mrakovic and Haley, 1979), cichlids (Winemiller and Taylor, 1982; Soliman *et al.*, 1986; Wimberger, 1993; Tave and Handwerker, 1994), salmonoids (Jensen, 1988; Harris and Hulsman, 1991), ictalurids (Lim and Lovell, 1978), and sparids (Koumoundouros *et al.*, 1997). It is also known in wild fishes (Valentine and Bridges, 1969; Lindesjöö *et al.*, 1994). Although the condition has been etiologically related to nutritional factors, particularly dietary ascorbic acid (vitamin C), other causes or contributing factors have been posited (e.g., pollutants, behavior, water current, water gas supersaturation). Not surprisingly, affected individuals exhibit poor growth/survival and increased disease susceptibility.

Mair (1992) found that opercular deformities were sometimes associated with a lethal condition known as caudal deformity syndrome (CDS) in *Tilapia* (=*Oreochromis*) *niloticus*. CDS is known to be under the control of an autosomal recessive gene, thus, more likely to occur in inbred tilapia. However, Tave and Handwerker (1994) demonstrated by breeding studies that OCD, when not associated with CDS, was non-heritable in *O. niloticus*. OCD presents after 4-5 generations of inbreeding in *Cichlasoma nigrofasciatum* (Winemiller and Taylor, 1982) and *Brachydanio rerio* (Mrakovic and Haley, 1979), suggesting that there is an underlying genetic cause in these two fishes. In captive breeding at OI, the condition appears in F1 progeny of

parental broodstock that are obtained directly from the wild population. If an underlying genetic factor is responsible for OCD in cultured threadin, then stocking progeny groups that contain affected hatchlings represents a potential Type II genetic hazard. Here, we report the result of a genetic assay in which the relationship between opercular deformity and maternal relatedness was investigated.

Two OI spawning groups, each composed of approximately 15 sires and dams, respectively, produced fertilized eggs in mass spawning events. Zygotes from these two spawning groups were mixed in approximately equal proportions, divided equally into four tanks, and reared for 25 days. Hatchlings from all four tanks were pooled into two nursery tanks and pooled again into one group prior to size gradation. From this mixed hatchling group, 24 specimens having deformed opercles and 25 developmentally normal specimens were collected for DNA analysis. Because OI broodstock are obtained from wild stock, inbreeding coefficients (levels of relatedness) among breeding pairs and among pairs of dams were assumed to be low. Haplotypes of potentially contributing females are as yet unknown; maternity was inferred from mtDNA control region haplotypes of hatchlings. The haplotype diversity level observed in the survey of wild threadfin suggests that the preponderance of OI female broodstock, which are collected from the wild, will have unique haplotypes. Taq error, inherent in the cloning step of DNA sequencing, may have caused a few maternally related individuals to be scored as unrelated; however, this phenomenon would be expected to occur randomly between deformed and normal hatchlings.

If there were no Taq errors, then at least 21 different dams contributed to the overall hatchling group, although contributions from other females may have been missed due to binomial sampling error. Relative maternal contributions to the sample of 49 hatchlings varied from 1 to 22 offspring per dam. Of the 22 hatchlings that shared one of the dams, 11 were deformed and 11 were normal. In 2 of the 3 other cases for which multiple individuals shared a particular dam, both normal and deformed hatchlings were observed. Results for the pairwise comparisons of cultured individuals (*described in* Material and Methods) are as follows: both deformed/related maternally – 56; both deformed/ unrelated maternally – 244; both normal/ related maternally – 84; both normal/ unrelated maternally – 192. If the deformity were positively associated with maternal relatedness, then we would expect the proportion of deformed individuals that shared a dam to be greater than the proportion of normal individuals that shared a dam. If the condition occurred randomly with respect to maternal relatedness, then the expected (null) proportion for both groups would be 50%. There appeared to be no positive association between the deformity and maternity. That is, among pairs of individuals that shared a dam, both individuals were deformed in 40% (56/140) of the comparisons and both were normal in 60% (84/140) of the comparisons. There was not a statistical difference between the above proportions and the expectation (V=2.79; $P > 0.1$ for both tests). Thus, these preliminary results offer no evidence that inbreeding and maternal effects were causes for the defect. However, the results could have been confounded if some of the contributing dams shared haplotypes.

We cannot fully dismiss the possibility that OCD has an underlying genetic cause. However, our data shed light on the potential for genetic impact on wild threadfin from stocking if the most common genetic factor – autosomal monogenic inheritance – has a role. *Penetrance* refers to the proportion of individuals that show an expected phenotype under a specific set of environmental conditions. *Expressivity* refers to the range of phenotypes expressed under a set of environmental conditions or over a range of environmental conditions. If genetically controlled, it is possible that a trait such as OCD will exhibit variable penetrance and/or expressivity – e.g., low penetrance/expressivity under wild conditions and high penetrance/expressivity during

captive breeding/rearing. Table 3 outlines phenotypic expectations for a hypothetical OCD variant for several forms of autosomal monogenic inheritance modes over a range of environmentally controlled penetrance values. We may generate several inferences using this table, our maternity data, and field observations of wild threadfin. OCD has not been observed in wild threadfin populations; thus, the morphological condition was neither expected nor apparent in any OI broodstock. Yet progeny of at least 13 different dams (more than one half of putatively contributing dams) were affected by OCD. If the trait were under the control of a recessive allele, then a very large proportion (approximately half) of the contributing dams (and one or more sire) would have had to have been heterozygous or phenotypically normal homozygous carriers of that allele. This would undoubtedly require that the allele be common in the wild population from which the broodstock were collected. If the allele were common in wild threadfin, the phenotypic expression of it would be not uncommon unless natural penetrance/expressivity was extremely low. Similarly, the various hypothetical dominance modes of gene action would also require naturally low penetrance/expressivity because the condition presents itself recurrently at OI in F1 progeny of phenotypically normal broodstock obtained directly from the wild population. More complex genetic mechanisms, e.g., certain sex-linked dynamics, epistatic interactions, and polygenic inheritance, cannot be ruled out without controlled breeding experiments.

Table 3. Phenotypic expectations for various modes of single-locus gene action for a hypothetical gene (*o*) that controls expression of opercular complex deformity. Wild-type alleles are designated by (+). The letter "p" represents the proportion of individuals of the specified genotype that exhibit the expected phenotype for a given set of environmental conditions. "Variable p" indicates that some form of expression occurs but varies in frequency of occurrence according to p.

Genotype	Penetrance of *o*		
	p = 100%	100% > p > 0%	p = 0%
A. Recessive *o*			
+ +	normal	normal	normal
+ *o*	normal	normal	normal
o o	deformed	deformed-variable p	normal
B. Dominant *o*			
+ +	normal	normal	normal
+ *o*	deformed	deformed-variable p	normal
o o	deformed	deformed-variable p	normal
C. Incompletely Dominant *o* (including additive)			
+ +	normal	normal	normal
+ *o*	partially deformed	partially deformed-variable p	normal
o o	deformed	deformed-variable p	normal
D. Incompletely Dominant Lethal *o*			
+ +	normal	normal	normal
+ *o*	deformed	deformed-variable p	normal
o o	dead	dead/deformed-variable p	normal

Selection acts on phenotypes. Thus, if OCD is under some form of simple autosomal genetic control for which there is low natural penetrance/expressivity, then the selection coefficient for the presumptive OCD gene in wild threadfin is expected to be very low unless the natural environment changes to a state that favors expression. From the standpoint of stock enhancement, this represents a low-risk scenario because the potential genetic impact, if any, would be purifying effect – captive breeding would expose individuals with OCD genotypes to selection. Reductions in allelic diversity at tightly linked gene loci would accompany this effect only if the wild population was swamped by stocked individuals. From the standpoint of other forms of threadfin aquaculture that may develop (e.g., commercial pond, cage, and net-pen culture), the above scenario, to the extent that the condition affects production and profitability, is somewhat more problematic. Mitigating the impact in this case would require either broodstock domestication or identification and elimination of exogenous factors that foster OCD expression.

To improve upon our initial experiment and eliminate sources of bias that may have been present, it is recommended that the experiment be replicated with samples from 2-3 additional progeny groups and include individual classification of degree of deformity in progeny. Left and right opercles of contributing broodstock should be retained for phenotypic evaluation as these individuals are cycled out of production. Markers that permit determination of both male and female parentage (e.g., 3-4 microsatellite loci) should be utilized if possible. Broodstock should be genotyped using finclips for DNA. If the condition remains randomly distributed among related and unrelated OI hatchlings within progeny groups and ubiquitous with respect to parental involvement, then critical data pertaining to reproductive variance and effective sizes of OI cohorts would be obtained simultaneously. Data pertaining to family size variance in OI progeny groups are needed to address Type III genetic concerns.

Concluding Remarks

From the standpoint of genetic management, the most significant finding in this preliminary genetic survey was that Pacific threadfin along (at least) the majority of the Hawaiian Island chain appear to comprise a single genetic stock. Maintenance of a larger gene pool via reproductive exchange between island populations may afford Pacific threadfin a degree of protection against the effects of drift and localized demographic stochasticity. Should the study of additional wild specimens support this finding, then the Type I genetic hazards identified by Tringali and Leber (1999) can be safely mitigated through the use of Hawaiian broodstock for threadfin stock enhancement, provided that impacts from Type II and III hazards are managed. Moreover, these Type I considerations can be extended to other forms of threadfin aquaculture that may occur in Hawaii, e.g., commercial pond, cage, and net-pen culture, particularly as they pertain to the issue of escapement. It will be important to ultimately resolve the evolutionary size and age of the Hawaiian threadfin population via outgroup sampling. If it turns out that threadfin colonized the island chain approximately 10^3 generations ago and maintained historically large effective sizes during that period, then the present population should not be overly burdened by segregating deleterious mutations (Lande, 1994), although that would require confirmation *via* crossbreeding studies. Nonetheless, given the current low abundances at some locations within the Hawaiian threadfin population and an overall low level of genetic variation putatively associated with a recent founding, maintenance of natural allelic

composition and adaptive diversity during large scale stocking efforts will require attention to broodstock numbers (N_e of cohorts) and post-stocking hatchery/wild ratios.

Acknowledgments

Literature Cited

Ballard, J. W. O. and M. Kreitmann. 1995. Is mitochondrial DNA a strictly neutral marker? *Trends Ecol. Evol.* 10:485-488.

Chubb, A. L., R. M. Zink, and J. M. Fitzsimons. 1998. Patterns of variation in Hawaiian freshwater fishes: The phylogeographic consequences of amphidromy. *J. Hered.* 89:8-16.

Cuvier, G. L. and A. Valenciennes. 1831. Histoire naturelle des poissons, vol. 7. Levrault, Paris.

DeSalle, R., A. Templeton, I. Mori, S. Pletscher, and J. S. Johnston. 1987. Temporal and spatial heterogeneity of mtDNA polymorphisms in natural populations of *Drosophila mercatorum*. *Genetics* 116:215-233.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* 131:479-491.

Fricke, R. 1999. Fishes of the Mascarene Islands (Réunion, Mauritius, Rodriguez): an annotated checklist, with descriptions of new species. Koenigstein (Koeltz Scientific Books), viii + 759 pp.

Friedlander, A. M. and D. A. Ziemann. Impact of hatchery releases on the recreational fishery for Pacific threadfin (*Polydactylus sexfilis*) in Hawaii. *Fish. Bull.*, in press.

Fu, Y. -X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking, and background selection. *Genetics* 147:915-925.

Fujii, T. 2002. Tracking released Japanese flounder, *Paralichthys olivaceus*, by mitochondrial DNA sequencing. UJNR this issue.

Gallagher, S. R. 1994. Commonly used biochemical techniques. Pp. A3.10-A3.11 In: K. Janssen (ed.) Current Protocols in Molecular Biology. John Wiley and Sons, Inc., USA.

Garber, A. M. 2001. Utilization of a hypervariable region as a molecular marker for red snapper, *Lutjanus campechanus*, stock enhancement. MS Thesis. The University of Southern Mississippi, Hattiesburg, MS. 124 pp.

Graves, J. E. 1998. Molecular insights into the population structure of marine fishes. *J. Heredity* 89:427-437.

Harpending, R. C. 1994. Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. *Hum. Biol.* 66:591-600.

Harris, K. C. and P. F. Hulsman. 1991. Intensive culture of lake whitefish (*Coregonus clupeaformis*) from larvae to yearling size using dry feeds. *Aquaculture* 96:255-268.

Higgins, D. G. and P. Sharp. 1988. Clustal: A package for performing multiple sequence alignment on a microcomputer. *Gene* 73:237-244.

Jensen, J. O. T. 1988. Combined effects of gas supersaturation and dissolved oxygen levels on steelhead trout (*Salmo gairdneri*) eggs, larvae, and fry. *Aquaculture* 68:131-139.

Koumoundouros, G., G. Oran, P. Divanach, S. Stefanakis, and M. Kentouri. 1997. The opercular complex deformity in intensive gilthead sea bream (*Sparus aurata* L.) larviculture. Moment of apparition and description. *Aquaculture* 156:165-177.

Kuhner, M. K., J. Yamato, and J. Felsenstein. 1995. Estimating effective population size and neutral mutation rate from sequence data using Metropolis-Hastings sampling. *Genetics* 140:1421-1430.

Kumar, S., K. Tamura, I. B. Jakobsen, and M. Nei. 2001. MEGA2: Molecular Evolutionary Genetics Analysis software, Arizona State University, Tempe, Arizona, USA.

Lande, R. 1994. Risk of population extinction from fixation of new deleterious mutations. *Evolution* 48:1460-1469.

Lim, C. and R. T. Lovell. 1978. Pathology of the vitamin C syndromes in channel catfish (*Ictalurus punctatus*). *J. Nutr.* 108:1137-1146.

Lindesjöö, E., J. Thulin, B. E-. Bengtsson, and U. Tjaernlund. 1994. Abnormalities of a gill cover bone, the operculum, in perch *Perca fluviatilis* from a pulp mill effluent area. *Aquat. Toxicol.* 28:189-207.

Mrakovic, M. and L. E. Haley. 1979. Inbreeding depression in the zebrafish *Brachydanio rerio* (Hamilton and Buchanan). *J. Fish Biol.* 15:323-327.

Mair, G. C. 1992. Caudal deformity syndrome (CDS): an autosomal recessive lethal mutation in the tilapia, *Oreochromis niloticus* (L.). *J. Fish Dis.* 15:71-75.

Merilä, J., M. Björklund, and A. J. Baker. 1997. Historical demography and present day population structure of the greenfinch, *Carduelis chloris* – an analysis of mtDNA control-region sequences. *Evolution* 51(3):946-956.

Mishra, S. S., S. Krishnan. 1993. *Polydactylus konadaensis* a new threadfin fish from India with a key to species of the Polynemidae of India. *Rec. Zool. Surv. India* 92:285-291.

Motomura, H. Y. Iwatsuki, S. Kimura, and T. Yoshino. 2000. Redescription of *Polydactylus macrochir* (Günther 1867), a senior synonym of *P. sheridani* (Macleay 1884) (Perciformes: Polynemidae). *Ichthyol. Res.* 47:327-333.

Motomura, H. Y. Iwatsuki, and S. Kimura. 2001. Redescription of *Polydactylus sexfilis* (Valenciennes in Cuvier and Valenciennes, 1831), a senior synonym of *P. kuru* (Bleeker, 1853) with designation of a lectotype (Perciformes: Polynemidae). *Ichthyol. Res.* 48:83-89.

Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York, NY, USA.

Nei, M. and W. H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci.* USA 76:5269-5273.

Paumbi, S. R. 1996. Nucleic acids II: The polymerase chain reaction. Pp. 205-247 <u>In</u>: D. M. Hillis, C. Moritz, and B. K. Mable (eds.) Molecular Systematics (2nd Edition). Sinauer Associates, Inc., Sunderland, Massachusetts USA.

Raymond, M. and R. Rousset. 1995. An exact test for population differentiation. *Evolution* 49:1280-1283.

Reeb, C. A. and B. A. Block. 2000. Structure and migration corridors in Pacific populations of the swordfish *Xiphius gladius*, as inferred through analysis of mitochondrial DNA. *Mar. Biol.* 136:1123-1131.

Rogers, A. R. 1995. Genetic evidence for a Pleistocene population explosion. *Evolution* 4 9:608-615.

Rogers, A. R. and H. C. Harpending. 1992. Population growth makes waves in the distribution of pairwise genetic distances. *Mol. Biol. Evol.* 9:552-569.

Saitou, N. and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4:406-425.

Schneider, S. and L. Excoffier. 1999. Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: Application to human mitochondrial DNA. *Genetics* 152:1079-1089.

Schneider, S., D. Roessli, and L. Excoffier. 1999. Arlequin version 2.0: A software for population genetic data analysis. Genetics and Biometry Lab, Department of Anthropology, University of Geneva, Switzerland. http://lgb.unige.ch/arlequin/.

Sekino, M., M. Hara, and N. Taniguchi. 2002. Genetic diversity within and between hatchery populations of Japanese flounder assessed by means of microsatellite and mitochondrial DNA sequencing analysis. UJNR this issue.

Seyoum, S., M. D. Tringali, T. M. Bert, D. McElroy, and R. Stokes. 2000. An analysis of genetic population structure in red drum, *Sciaenops ocellatus*, based on mitochondrial DNA control region sequences. *Fish. Bull.* 98:127-138.

Slatkin, M. 1991. Inbreeding coefficients and coalescence times. *Genet. Res. Camb.* 58:167-175.

Soliman, A. K., K. Jauncey, and R. H. Roberts. 1986. The effect of varying forms of dietary ascorbic acid on the nutrition of juvenile tilapias (*Oreochromis niloticus*). *Aquaculture* 52:1-10.

Springer, V. 1982. Pacific plate biogeography, with special reference to shorefishes. Smithson. Contr. Zool. No. 367. 182 pp.

Stepien, C. 1995. Population genetic divergence and geographic patterns from DNA sequences: Examples from marine and freshwater fishes. *Am. Fish. Soc. Symp.* 17:263-287.

Taggart, J. B., R. A. Hynes, P. A. Prodhol, and A. Ferguson. 1992. A simplified protocol for routine total DNA isolation from salmonid fishes. *J. Fish Biol.* 40:963-965.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123:585-595.

Tamura, K. and M. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.* 10:512-526.

Tave, D. and T. S. Handwerker. 1994. Semi-operculum: a non-heritable birth defect in *Tilapia nilotica*. *J. World Aquac. Soc.* 25(2):333-336.

Tringali, M. D. and T. M. Bert. 1998. Risk to effective population size should be an important consideration in fish stock enhancement programs. *Bull. Mar. Sci.* 62(2):641-660.

Tringali, M. D. and K. M. Leber. 1999. Genetic considerations during the experimental and expanded phases of snook stock enhancement. *Bull. Natl. Res. Inst. Aquacult., Suppl.* 1:109-119.

Tringali, M. D., T. M. Bert, S. Seyoum, E. Bermingham, and D. Bartolacci. 1999. Molecular phylogenetics and ecological diversification of the transisthmian fish genus *Centropomus* (Perciformes: Centropomidae). *Mol. Phylogen. Evol.* 13(1):193-207.

Valentine, D. W. and K. W. Bridges. 1969. High incidence of deformities in the serranid fish *Paralabrax nebulifer* from southern California. *Copeia* 3:637-638.

Wilson, A. C., R. L. Cann, S. M. Carr, M. George, U. B. Gyllensten, K. M. Helm-Bychowski, R. G. Higuchi, S. R. Palumbi, E. M. Prager, R. D. Sace, and M. Stoneking. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. *Biol. J. Linn. Soc.* 26:375-400.

Wimberger, P. H. 1993. Effects of vitamin C on body shape and skull osteology in *Geophagus brasiliensis*: Implications for interpretations of morphological plasticity. *Copeia* 2:343-351.

Winemiller, K. O., and D. H. Taylor. 1982. Inbreeding depression in the convict cichlid, *Cichlasoma nigrofasciatum* (Baird and Girard). *J. Fish Biol.* 21:399-402.

Ziemann, D. A. 2002. Stock enhancement of the Pacific threadfin (*Polydactylus sexfilis*) in Hawaii: Progress and currrent status. UJNR, this issue.

Ziemann, D. A. and A. M. Friedlander. Carrying capacity for hatchery-reared Pacific threadfin (*Polydactylus sexfilis*) in native nursery habitats in Hawaii. *Trans. Amer. Fish. Soc.*, in press.

Enhancement of Pacific Threadfin *Polydactylus sexfilis* in Hawaii: Interactions Between Aquaculture and Fisheries

David A. Ziemann
Oceanic Institute
41-202 Kalanianaole Highway
Waimanalo, Hawaii 96795
USA

Abstract

Many coastal fisheries in the Hawaiian Islands show evidence of depletion through over fishing or loss of critical habitat. While conventional stock management (imposition of harvest controls) may aid some over fished stocks to recovery, generally such recovery is slow and subject to variability of natural recruitment. Research into the feasibility of stock enhancement (release of hatchery-reared fish to supplement stocks and reproductive success) in Hawaii on Pacific threadfin (moi, *Polydactylus sexfilis*) has established the information necessary to design and implement a responsible enhancement program, and has demonstrated the potential contribution released fish can have on localized fisheries. Current research is examining threadfin behavior and conditioning, fisheries demographics and ecology, genetics, the ecological basis of recruitment success and means to determine the contribution of hatchery-reared fish to reproduction and stock recovery.

Introduction

In Hawaii, as in other locations around the world, local fishery yields have leveled off or are decreasing (Shomura, 1987). Many stocks are over exploited, fully exploited or of questionable status. While the state Division of Aquatic Resources, the responsible management agency, collects catch data for holders of commercial fishing licenses, most of these fishers target offshore pelagic or demersal fisheries. The state does not license recreational or subsistence fishers, nor does it collect catch data from shoreline fishers who target coastal and inshore fisheries.

Resource managers have a range of options for managing depleted fisheries stocks. For growth-limited fisheries, where fishery yield is less than maximum sustainable yield due to over fishing, managers typically enact regulations to restrict fishing effort, either through catch limits, size limits, gear restrictions, closed seasons or some combination. Recently, managers are examining the effectiveness of establishing natural reserves, or implementing community-based management for localized fisheries.

Recruitment-limited fisheries are those whose rate of natural reproduction and/or recruitment is less than that needed to maintain the population at optimal levels. The limit to reproduction is primarily due to severely depleted adult (reproductive) stocks, loss of spawning or nursery habitat, or both. In such cases, even complete bans on the taking of these stocks may not result in recovery, or recovery may be extremely slow because the net rate of increase of the population is low, or inter-annual variations in recruitment result in only sporadic strong year

classes. For recruitment-limited stocks, management options include increasing recruitment through propagation and release, most commonly of competent juveniles, but potentially of mature adults as well, or restoring degraded spawning and nursery habitat.

Coastal fisheries in Hawaii are sensitive to natural variations in environmental conditions, particularly rainfall and runoff and high surf events, as well as man-made environmental perturbations such as coastal dredging and sedimentation from terrestrial sources. The impacts of these perturbations most strongly influence early survival, and are one of the primary factors affecting inter-annual variations in recruitment success.

The Pacific threadfin, *Polydactylus sexfilis*, is one of the most culturally important, locally popular coastal fish species in Hawaii. *P. sexfilis* is a member of family Polynemidae, comprising 33 species with tropical and subtropical distributions. Pacific threadfin in Hawaii is known as moi, the "fish of kings."

Pacific threadfin is an ideal candidate for a stock enhancement program. The Pacific threadfin fishery in Hawaii is highly depleted, and in response, there are regulations setting limits on the daily catch, minimum size, and a closed season. All available evidence suggests Pacific threadfin is recruitment limited, at least on the island of Oahu. Culture techniques for the species are well established. The fish spawn spontaneously in captivity, produce large numbers of healthy fry, and can be grown to a size appropriate for tagging within 60-90 days after hatch. The juveniles inhabit defined nursery habitats, high wave energy sandy beaches, while the adults move offshore to sand patches in hard bottom areas.

The Oceanic Institute has been conducting research into the enhancement of depleted fisheries for over a decade, currently focusing on Pacific threadfin, an important but depleted coastal fishery in Hawaii. Our capability at the Institute to produce large numbers (100,000 per month) of healthy fry on a continuous basis, combined with Hawaii's advantages of year-round warm temperatures, unpolluted coastal waters and undegraded habitats, form the foundation for innovative enhancement research.

Early research on Pacific threadfin focused on the determination of optimal release strategies (size, site and season) (Leber *et al.*, 1998). Having established a capability to produce, tag and release large numbers of fry with reasonable return rates, the focus of our enhancement research has turned from "can we release and recapture fish" to "how do we conduct enhancement responsibly?"

Research Components

Eight primary areas of research for Pacific threadfin stock enhancement development have been identified for focus by the Hawaii Stock Management (HSM) Program at the Oceanic Institute.

These are culture technology; release optimization; fisheries demographics, ecological interactions and habitat utilization; behavior and conditioning; health management; genetic management; economic considerations; and the ecological basis of fishery production. All research has been conducted along the windward coast of the island of Oahu (Fig.1).
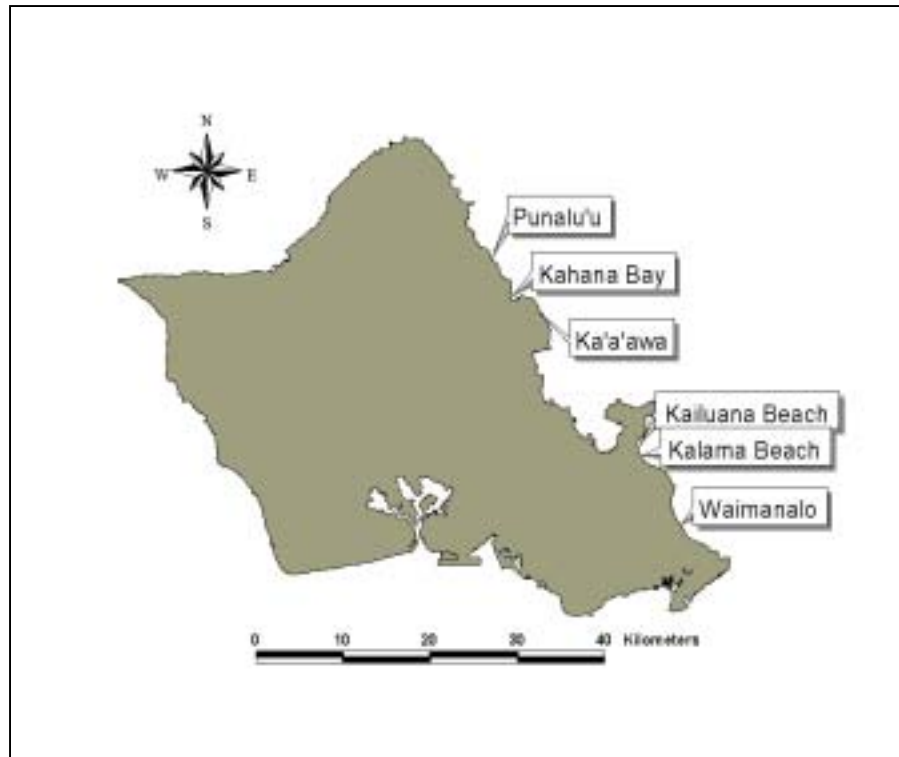
Figure 1. Map of Oahu, Hawaii, showing locations of primary sampling sites.

Culture and Tagging Technology

The culture of moi at the Oceanic Institute (OI) is described by Ostrowski *et al.* (1996). In brief, production runs began with the spawning of wild broodstock maintained at OI. OI maintains a large (~100) population of wild-caught broodstock, from the general areas in which release experiments are conducted. Larval rearing requires 25-30 days, depending on temperature. Larvae receive formulated and live food (rotifers, *Artemia* nauplii); juveniles receive Moore-Clark pellet feed. Survival rates are approximately 30% during the larval period, 70-80% during stage-1 nursery, and >90% after Day 40. Most fish are released into coastal waters between Day 60 for small juveniles (70-85 mm, fork length) and Day 90 for larger juveniles (130-150 mm).

Before release, all fish receive coded wire tags (cwt, Northwest Marine Technologies) in the snout area to identify a release batch of the same size fish, date and location. To determine tag retention rates, approximately 5% of each release lot are retained and examined monthly for one to six months, until the tag loss rate stabilizes (i.e., when the number lost had not increased since the previous month). Tag retention rates vary from 92.3% to 99.2%.

Release Optimization

The HSM Program conducted a multi-year study to examine the recapture rates of hatchery-reared moi released into Kahana Bay, Oahu from 1996-1999, focusing on the influence size at release had on recapture success (Ziemann *et al.,* in prep). Release experimental design was based on patterns of natural recruitment. Releases were

conducted primarily in fall and winter, the peak season for wild recruitment to the sandy beach nursery habitats. Releases and recapture sampling focused on the sandy beach habitat, which is known to be the preferred nursery habitat. Fish were sorted into four size classes and coded-wire tagged for size, location, season and replicate batch. Release size classes ranged from 70 mm, the minimum size to safely handle and tag fingerlings, to 130 mm.

Two types of recapture methods were used: beach seining and recovery of fish from commercial and recreational fishermen through the use of a creel survey and reward program. Results varied from year to year, but some trends were evident. Because the release experiments patterned releases after known patterns of natural recruitment, and thus eliminated extremes of size, out of season releases, or releases into unsuitable habitat, no major effects of size, site or season were observed. One statistically significant difference was distinguished between beach seine recapture percentages for two size classes in the 1997 releases, in which 85-100 mm FL moi were recaptured at about twice the rate of 100-115 mm FL moi. In 1997, the release period extended for two seasons of the year, allowing for a within-year comparison of summer and fall releases. For that year, smaller fish appeared to survive better than larger fish in both summer and fall, but recapture rates were slightly higher for summer releases than for fall releases. Based on these results and similar ones from a release in 1994, an optimal release strategy for Kahana Bay may be either to release small fish in the summer months or large fish in the winter.

Fisheries Demographics and Ecology

Contribution to the Recreational Fishery   ⊦   The HSM Program conducted a multi-year study to examine the contribution of hatchery-reared fish to the recreational fishery along the windward coast of Oahu, Hawaii (Friedlander and Ziemann, 2003). Over 340,000 fingerlings of various sizes were implanted with coded wire tags and released in nursery habitats along the windward coast of Oahu between 1993 and 1997. Because few Pacific threadfin were present in creel surveys conducted between 1994 and 1998, Oahu fishermen were offered a $10 reward for each threadfin (hatchery-reared and wild) caught. A total of 1,882 Pacific threadfin were recovered from the reward program between March 1998 and May 1999, including 163 hatchery-reared fish, an overall contribution of 8.7% to the fishery. Hatchery-reared fish were as high as 71% of returns in the release areas. Hatchery-reared fish were recovered on average 11.5 km (SD=9.8 km) from the release site, though some had moved as far away as 42 km. Average age for recovered hatchery-reared fish was 495 days with the oldest being 1,021 days.

Cultured Pacific threadfin juveniles survived and recruited successfully to the recreational fishery, accounting for 10% of fishermen's catches on the windward side of Oahu. Recruitment to the fishery was highest for the 1997 release year; few juveniles from earlier releases were observed. Presence of a few large, fully-developed females in the recreational fishery suggests hatchery-reared fish can survive, grow and reproductively contribute to the population.

Figure 2. Mean monthly catch per unit effort (CPUE) for year 0 Pacific threadfin in nursery habitat on the windward coast of Oahu.

Monitoring Natural Recruitment - The HSM Program conducted monthly beach seine surveys continuously over a five-year period (1997-2001) at six nursery habitat beaches along the windward coast of the island of Oahu (Ziemann, in prep). The beach seine measured 24 x 1.8 m with 1.3 cm mesh. During each sampling period, a consistent level of effort was followed.

Sampling efforts consisted of a series of 6 to 12 seine hauls, depending on the length of sandy beach available; data were standardized to catch per unit effort (CPUE=number of fish caught per haul). Hauls were started at a distance of about 10 m offshore or in a water depth of about 1-1.5 m and pulled directly towards shore. Sampling generally occurred during mid-tidal heights in morning but without specific regard to tidal height, times of day, or weather condition. Sampling was only conducted when surf height along the shoreline was 0.6 m or less.

The CPUE for wild threadfin juveniles collected during the recruitment study is presented in Fig. 2. Several patterns are evident. First, for all years, peak recruitment was observed during winter months (November - January), with low or no recruitment observed during summer months. Second, the inter-annual variability in overall recruitment was large, with highest monthly levels observed in 1997, and lowest levels observed in 2001. Finally, the extremely low levels observed in 2001 suggest that the particular combination of low adult population size and apparently poor larval survival resulted in almost total failure of the 2001 recruitment year class.

Habitat Utilization in Movement patterns and habitat utilization of moi (*Polydactylus sexfilis*) were assessed using data from tag-and-release studies and acoustic tracking (Friedlander and Ziemann, in press). The locations and dates of capture for each fish returned in the recreational fishery survey were analyzed to calculate net displacement (distance from release site). Long-term movement of over 60 km occurred along the windward coast of Oahu (Fig. 3).

The sandy surf zone habitat at the Kahana Bay and Kailua Bay release sites provided good juvenile habitat while rocky high wave energy habitats such as Mokapu Peninsula and Kahuku provided better habitat for larger individuals. The smallest size class released (70-85 mm FL) had the greatest number of days at liberty and the longest range of movement. The smaller movement associated with larger size classes may be owing to their susceptibility to exploitation soon after release.

Acoustic tracking of small (150-170 mm FL) hatchery-reared moi was

Figure 3. Location of collection and percent contribution of cultured fish released at Kahana Bay in 1997 to the recreational fishery. A: 1998, B: 1999.

conducted periodically over a two-year period (2000-2001). Hatchery fish were surgically implanted with a Vemco acoustic tag, stabilized for two days in a recovery tank, and released with 10-12 similar fish into nursery habitat in Kahana Bay or Kailua Bay. Fish were tracked continuously up to 48 hours after release with an acoustic receiver on a small boat. Position/bearing plots were recorded to estimate day and night habitat range and movement. Acoustic tracking showed limited movement along the sandy surf zone habitat during the day and increased activity at night with more movement offshore. Similar patterns were seen for most of the fish tracked.
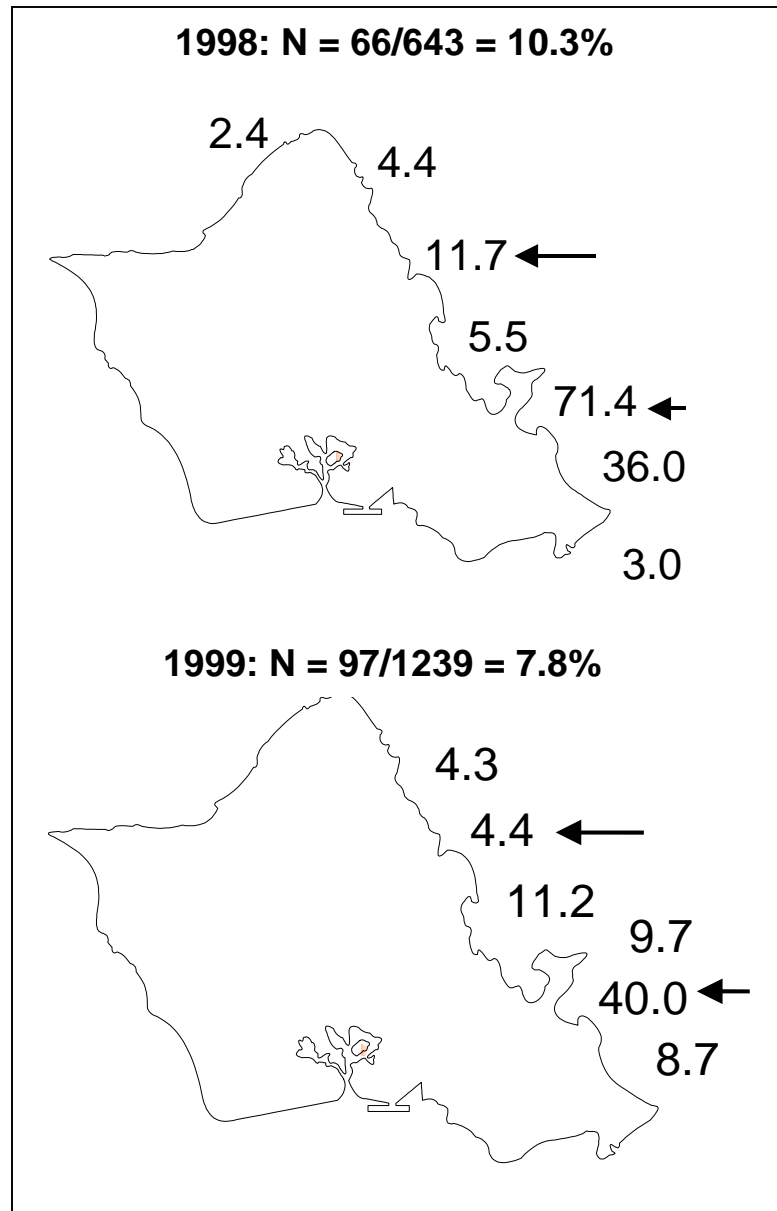
Diet and Feeding   ⊦   Because the diet of an organism has considerable influence upon survival and fitness, the acclimation of hatchery-reared fish to the natural diet is an important component to the success of a release program. Research examined the dietary composition of juvenile, subadult, and adult wild and cultured *P. sexfilis* from the coastal waters of east Oahu, Hawaii (Ogawa *et al.,* in prep). The intention of this study was to establish the major dietary characteristics of wild *P. sexfilis* and to make general dietary comparisons between hatchery-reared and wild *P. sexfilis*. The dietary characteristics of wild and hatchery-reared *P. sexfilis* captured from the east coast of Oahu, Hawaii were very similar. Small benthic crustaceans such as shrimps and amphipods dominated the diet of juvenile fish whereas shrimps, crabs, and fish were the predominant prey items found in adult fish. These prey items are typical among polynemids. Differences in diet were found among size classes for both cultured and wild fish. Horn's overlap index was used to qualitatively compare diets and the Mann-Whitney rank sum test used to detect differences between relative prey weights. Cultured fish feeding habits immediately after release were dissimilar from wild fish, but cultured fish diets rapidly changed to approximate those of wild fish (Fig. 4).
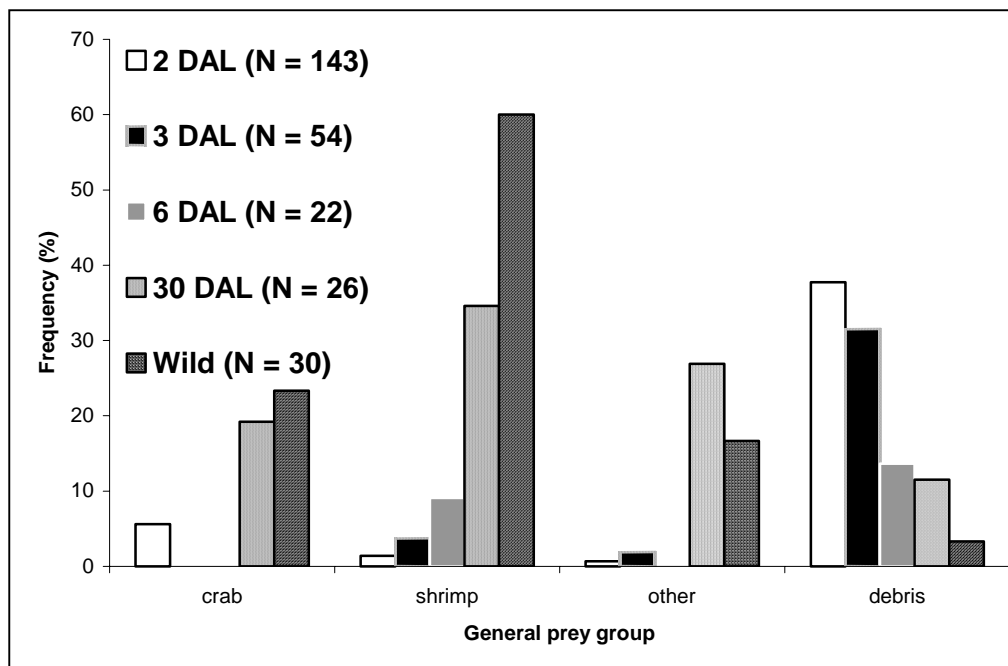

Figure 4. Primary food items found in stomachs of released Pacific threadfin at 2, 3, 6 and 30 days after release, and wild fish, collected in Kahana Bay.

Age and Growth   ⊦   Increased knowledge of early life history characteristics is needed to address fundamental questions of age and growth patterns of Pacific threadfin, which may be helpful in artificial propagation and larval rearing. The HSM Program conducted a study (Bloom *et al.*, submitted) to validate daily increment formation in otoliths of Pacific threadfin, and to conduct preliminary age determinations on wild juveniles. Wild juvenile Pacific threadfin were collected in Kahana Bay, Oahu, Hawaii, from December 1998 to May 1999. Saggitae were removed, cleaned of endolymph tissue, and stored dry in cell culture trays. Otolith microstructure of wild juveniles was examined. Successful validation of "daily" growth rings was established from cultured

juvenile fish of known age. Age estimations were made for wild juvenile Pacific threadfin (n=50). Growth rates for the initial 30 days of growth are reported for wild juvenile fish, along with changes in daily growth. For wild juveniles collected in 1999, back-calculated hatch days indicated that the year class was the product of multiple spawnings over a four month period in winter.

Behavior and Conditioning

Predation is hypothesized to be a major cause of post-release mortality in stock enhancement projects. We evaluated (Masuda and Ziemann, in press) critical size and release condition of *Polydactylus sexfilis* juveniles in regard to their ability to avoid potential predators such as bluefin trevally *Caranx melampygus* and hammerhead sharks *Sphyrina lewini*. Four different sizes (70, 100, 140 and 190 mm in FL) of threadfin juveniles were released into experimental tanks with predators (5-6 individuals of one of above species) after 24 hrs of acclimatization. Fish were released either gently (control group) or after the stress of 1-min air exposure (stressed group). When they encountered trevallies, fish released at 70 mm or 100 mm were eaten within 7 min 10 sec in maximum, whereas fish released at 140 mm or 190 mm survived for a minimum of 1 hr. There was no difference between stressed and control group. In the case of encountering sharks, there was no size dependent mortality, although individuals in the stressed group tended to be eaten more readily than individuals in the control group. For both predators heavy predation occurred only in the first hour after the release, suggesting that hatchery-reared fish can learn how to avoid predators in a relatively short period. In the practice of stock enhancement we suggest the importance of assessing critical size and species of potential predators before release.

Threadfin are known to spawn near the last quarter of moon phase both in the wild and under culture. Since they utilize the surf zone as their habitat and spawn corresponding to lunar rhythm, we expected that wild threadfin should have activity rhythm corresponding to tidal and lunar rhythm. Cultured threadfin, on the other hand, may or may not have such rhythm, or they may have specific rhythm corresponding to the feeding times in the rearing tanks. The aim of the circadian rhythm experiment (Masuda *et al.*, in press) was to examine the daily and lunar behavioral rhythm of wild and cultured threadfin. Behavior of both wild-caught and cultured fish were video-recorded simultaneously and analyzed. Field sampling and gut content analysis data was also included to extend indoor data to the field. Laboratory experiment suggested that threadfin is a nocturnal species, since both swimming speed and swimming depth was higher at night in both wild and cultured fish. This nocturnal activity may be related to either feeding behavior or migratory behavior. Since they are bottom feeders, off-bottom behavior observed at night may be related to migration rather than feeding. Both field sampling and laboratory experiments suggested that new moon nights would provide better condition for threadfin compared to other moon phases. Therefore it is recommended to release threadfin around new moon to improve the survival potential in the stock enhancement of this species.

Genetic Management

      Preliminary aspects of genetic management for Pacific threadfin stock enhancement research at the Oceanic Institute (OI) have been focused on genetic stock identification and broodstock management (Tringali *et al.*, this volume). To investigate genetic structure in threadfin populations potentially impacted by stock enhancement, wild specimens from four locations on Hawaii (*n*=41) and from three locations on Oahu (*n*=32) were assayed by sequencing 1045 base pairs of the mitochondrial DNA (mtDNA) control region. Overall, haplotype diversity was high (99.3%); a total of 61 unique haplotypes were observed from the 73 individuals assayed. However, nucleotide diversity was low (0.64%). No phylogeographic structure was evident in clustered haplotypes. Genetic variance was partitioned predominantly among individuals within populations (98%); approximately 1% of the genetic variance occurred between the threadfin from the islands of Oahu and Hawaii. Haplotype distributions did not differ significantly among these two locations. These data, which are preliminary, are suggestive of high gene flow on a regional basis. The female effective population size, estimated using a Maximum Likelihood Metropolis-Hastings sampling method, ranged approximately 200,000-400,000. The sampled population appears to have undergone a large, historical expansion. Taken together, data are consistent with an evolutionarily recent colonization of the species in the Hawaiian Islands. Preliminary studies for broodstock management are focusing on levels of relatedness among female broodstock and female contributions to OI progeny groups. Using mtDNA sequencing, maternity surveys were performed for cultured progeny groups which contained both normal individuals and individuals exhibiting a particular morpho-anatomical deformity. The condition appeared to be manifested ubiquitously and randomly among progeny of the contributing females. Data provided no evidence that inbreeding or maternal effects are causative factors. If controlled by a single autosomal (dominant or recessive) gene, our preliminary results suggest that the condition has very low penetrance/expressivity in the wild threadfin population.

Ecological Basis of Natural Recruitment

      Rates of natural recruitment of Pacific threadfin to nursery habitats has been observed to be highly variable between years (Fig. 2). Recruitment success is the result of the actions of a range of factors, both related to the number of larvae produced (size of the reproductive population, spawning frequency and success), and to environmental factors acting at the time of spawning and the early larval stage (low predator abundance, high food availability, favorable physical conditions). Preliminary data collected by the HSM Program suggests a relationship between natural recruitment and environmental factors (Fig 5; Ziemann and Friedlander, in press); in this case, mean annual temperature may be a proxy indicator of overall rainfall, which influences the input of dissolved nutrients into coastal waters.
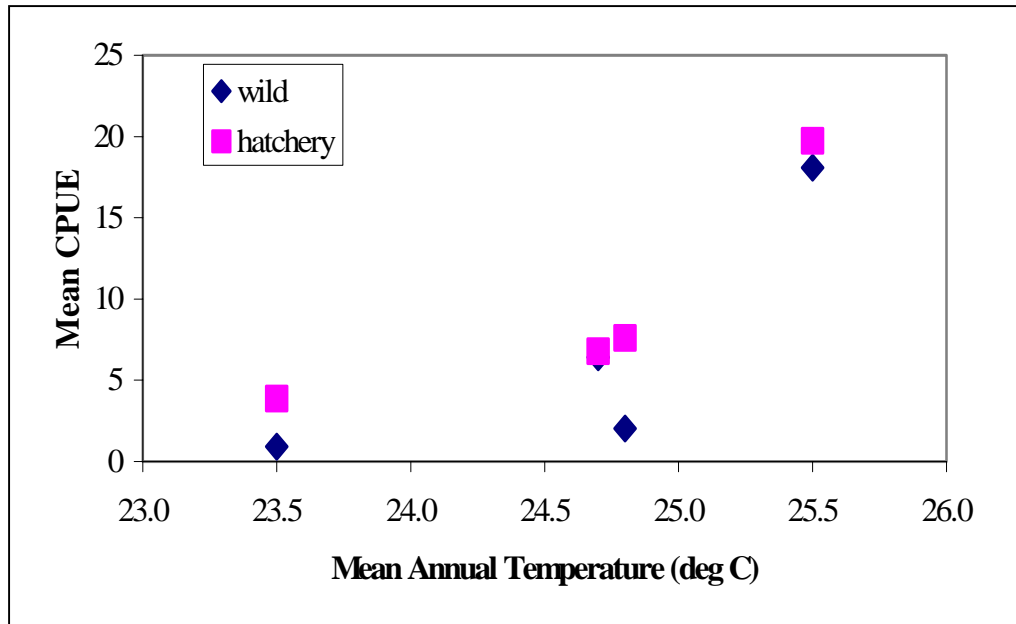
Figure 5. Plot of mean annual catch per unit effort for wild and released year 0 Pacific threadfin along the windward coast of Oahu, related to mean annual water temperature at Kahana Bay, Oahu.

The HSM Program has begun a research component to examine the ecological basis of natural recruitment in Pacific threadfin. The study entails monthly physical, chemical and biological surveys focused on Kahana Bay, a primary site for early threadfin recruitment. Physical oceanographic studies include measurements of currents, and determination of the impacts of tidal exchange on the distribution and concentrations of dissolved nutrients. Nutrient input studies are examining the sources and types of nutrients entering the bay, and generating estimates of uptake and dispersal rates. Biological studies are focusing on the distribution, abundance and major taxonomic components of the benthic and planktonic communities.

Summary

Research into the feasibility of stock enhancement in Hawaii on Pacific threadfin has established the information necessary to design and implement a responsible enhancement program, and has demonstrated the potential contribution released fish can have on localized fisheries. Our research has shown: the optimal release strategy matches natural recruitment patterns; cultured fish adapt quickly to natural conditions; and experimental releases have made significant contributions to Oahu recreational moi fishery. The threadfin population, on the island of Oahu, at least, is severely depleted, suffering from both low adult population size and low and variable recruitment. Research has shown significant interactions between adult population size, natural recruitment, and the impacts of releases. Current research is examining threadfin behavior and conditioning, fisheries demographics and ecology, genetics, the

ecological basis of recruitment success and means to determine the contribution of hatchery-reared fish to reproduction and stock recovery. Major questions on factors affecting population size, nursery carrying capacity and recruitment success, wild *vs.* hatchery fish interactions, and the long-term (multi-generational) effects of releases remain.

Acknowledgments

Literature Cited

Bloom, S. W. S., D. A. Ziemann, and T. Ogawa. Age determination of wild juvenile Pacific threadfin, *Polydactylus sexfilis,* in Hawaii using daily increment formation. *Trans. Am. Fish. Soc.* Submitted.

Friedlander, A. M. and D. A. Ziemann. 2003. Impact of hatchery releases on the recreational fishery for Pacific threadfin (*Polydactylus sexfilis*) in Hawaii. *Fish. Bull.* 101(1).

Friedlander, A. M. and D. A. Ziemann. Movement patterns and habitat utilization of hatchery-reared moi, *Polydactylus sexfilis,* in Hawaii. *Fish. Bull.*, in press.

Leber, K. M, N. P. Brennan, and S. M. Arce. 1998. Recruitment patterns of cultured juvenile Pacific threadfin, *Polydactylus sexfilis* (Polynemidae), released along sandy marine shores in Hawaii. *Bull. Mar. Sci.* 62:389-408.

Masuda, R. and D. A. Ziemann. Vulnerability of Pacific threadfin juveniles to predation by bluefin trevally and hammerhead shark: size dependent mortality and handling stress. *Aquaculture,* in press.

Masuda, R., C. Hirota, T. Ogawa, and D. A. Ziemann. Feeding mechanics of wild and hatchery-reared Pacific threadfin with emphasis on their searching capability and diel rhythm. *Aquaculture*, in press.

Ogawa, T. K., D. A. Ziemann, and S. W. S. Bloom. Feeding habits of wild and hatchery-reared Pacific threadfin (*Polydactylus sexfilis*) in Hawaii, in preparation.

Ostrowski, A., T. Iwai, S. Monahan, S. Unger, D. Dagdagan, P. Murawaka, A. Schivell, and C. Pigao. 1996. Nursery production technology for Pacific threadfin (*Polydactylus sexfilis*). *Aquaculture* 139:19-29.

Shomura, R. S. 1987. Hawaii's marine fishery resources: Yesterday (1900) and today (1986). National Marine Fisheries Service, Southwest Fisheries Center, Honolulu Laboratory. Administrative Report H-87-21.

Tringali, M. D., D. A. Ziemann, and K. C. Stuck. This volume. Preliminary aspects of genetic management for Pacific threadfin (*Polydactylus sexfilis*) stock enhancement research in Hawaii.

Ziemann, D. A., M. Dunlap, and A. M. Friedlander. Enhancement of Pacific Threadfin *Polydactylus sexfilis* in Hawaii: Release Optimization, in preparation.

Ziemann, D. A. and A. M. Friedlander. Carrying capacity for hatchery-reared Pacific threadfin (*Polydactylus sexfilis*) in native nursery habitats in Hawaii. *Trans. Am. Fish. Soc.,* in press.

Ziemann, D. A. Interannual patterns in recriutment of juvenile Pacific threadfin (*Polydactylus sexfilis*) to nursery habitat in Hawaii, in preparation.

Aquaculture and Genetic Structure in the Japanese Eel
Anguilla japonica

Masaya Katoh
Ishigaki Tropical Station
Seikai National Fisheries Research Institute
Fisheries Research Agency
Fukai-Ohta 148-446, Ishigaki, Okinawa 907-0451
JAPAN
Email: mkatoh@fra.affrc.go.jp

Masahiro Kobayashi
Fisheries Agency
Kasumigaseki 1-2-1, Chiyoda-ku, Tokyo 100-0013
JAPAN

Abstract

Both commercial catches of natural eel and eel seeds for aquaculture have decreased over four decades. These declines may indicate a severe biomass reduction of the Japanese eel (*Anguilla japonica*). Although the causes of this reduction are not clear, more knowledge is necessary of the basic biology of the Japanese eel, including knowledge of their migration and population dynamics and structure, which will contribute to resource management. We studied the genetic structure of *A. japonica* by the random amplified polymorphic DNA (RAPD) technique. Glass eel were collected from Taiwan, Kagoshima, Ibaraki, and Miyagi. The frequencies of most RAPD bands were similar among the four locations, but the frequencies of 1100bp band amplified by the Operon A10 primer showed nearly significant difference between Miyagi and the others. Additional genetic studies are needed to determine the population structure of the Japanese eel.

Introduction

The annual eel consumption in Japan currently amounts to 120,000 to 130,000 tons. In other words, the per capita consumption is about 5 eel a year. *Unajuu,* or *Unadon*, a dish of charcoal-grilled eel filets served with sweet soy sauce on rice, is as important in Japan as sushi. Eighteen percent of the eel consumed in Japan is produced in the country (23,211 tons, aquaculture; and 817 tons, wild in 1999), and the remainder is imported from China, Taiwan, and Malaysia. Only 0.6% of the total is wild adult eel. Therefore, eel aquaculture is very important to the success of the eel industry. The Prefectures of Aichi, Kagoshima, Shizuoka, and Miyazaki are major producers. However, the recent decline of glass eel catches in East Asia has caused serious problems in eel aquaculture in Japan and Taiwan. Although scientists at the National Research Institute of Aquaculture have reared hatched eel larvae for more than 250 days (Tanaka *et al.*, 2000), complete artificial propagation, from eggs to glass eel, has not been successful. At this time, seeds for aquaculture are totally dependent on wild glass eel from East Asia and Europe. This paper deals with the decline of natural eel resources and the genetic structure of the Japanese eel (*Anguilla japonica*).

Decline of Natural Eel Resources

The Japanese eel has been a major target species for aquaculture in East Asia, and there have been many studies of eel aquaculture and related topics, such as disease control, artificial maturation, propagation, and searches for spawning sites. However, little attention has been given to natural populations and the resource management of eel. Biomass is considered proportional to the catch per unit effort (CPUE). The CPUE in eel fisheries is not available in Japan. Available resource data come from yearly commercial catches of natural eel, which may have a correlation with the biomass. The annual catch of natural eel in the inland waters of Japan was around 3,000 tons between 1901 and 1941 (Matsui, 1952). According to statistics of the Ministry of Agriculture and Forestry and the Ministry of Agriculture, Forestry and Fisheries (name of the ministry since 1978), about 3,000 tons of natural eel were caught annually in the 1960s. However, the annual catches started to decline in 1970 to ca. 2,000 tons in 1979 (Fig. 1). The decline has continued in the 1980s and 1990s. Since 1993, the catch has been less than 1,000 tons a year. The one-third reduction in commercial catch of natural eel during the past four decades may imply a severe reduction of the eel biomass.
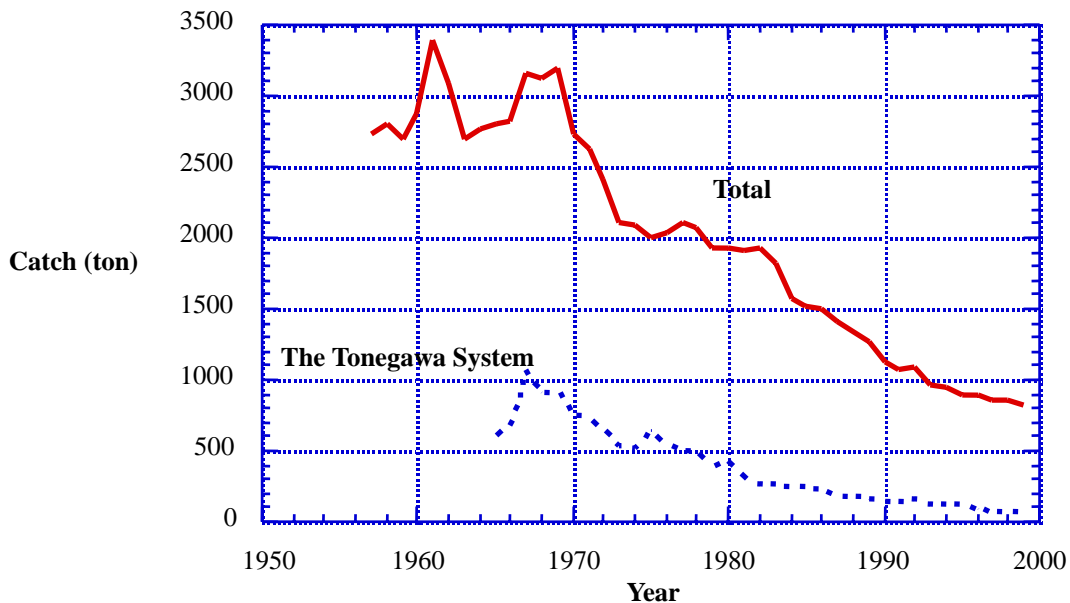

Figure 1. Annual catch of natural eel in Japan.

The annual commercial catch of natural seeds, which includes the glass eel, has decreased even more obviously. In statistics from the Ministry of Agriculture, Forestry and Fisheries of Japan, glass eel are listed in two categories: 1) the coastal catch for aquaculture, and 2) aquaculture seeds from the inland waterways, which include glass eel and juveniles up to one-year-old. Figure 2 shows the sum of the two categories, which is the annual catch of natural eel seeds for aquaculture in Japan. During the 1960s, up to 150 tons of natural eel seeds were caught annually. The catch ranged from 50 to 100 tons in the 1970s and declined to fewer than 50 in the 1980s. Currently, the catch has been around 20 tons (Fig. 2). The seed catch is one-fifteenth what it was 30 years ago. The reduction of the seed and natural eel catch indicates that the eel biomass has decreased.

The Tonegawa drainage system near the Tokyo Metropolitan area used to be known as a major production area for eel seeds. In 1958, a total of 179 tons of eel seeds were caught in the drainage system (Kasebayashi, 1960). The seed catch in the Tonegawa drainage was 76% of the total catch in 1969 (Fig. 2). These seeds were transported to Shizuoka and Aichi Prefectures for aquaculture. Fewer than 10 tons were caught in the Tonegawa drainage in 1999. Huge amounts of eel seeds used to be caught in the Tonegawa drainage. If the catch of eel seeds was the same today as it was forty years ago in the Tonegawa drainage alone, there would be no shortage of glass eel for aquaculture in Japan. Moreover, the natural eel catch in the Tonegawa drainage was one-third of the total in Japan several decades ago. Therefore, the Tonegawa drainage system used to be a major habitat for the Japanese eel, and it could possibly be used today to sustain eel resources. Kasebayashi (1960) suggested that the ongoing river development in the Tonegawa system might negatively affect the eel population. The reduction in the eel population coincided with the construction of dams in the lower reaches of the river. The construction might cause eutrophication of Lake Kasumigaura (220 km^2) because of the lower exchange between freshwater and seawater.
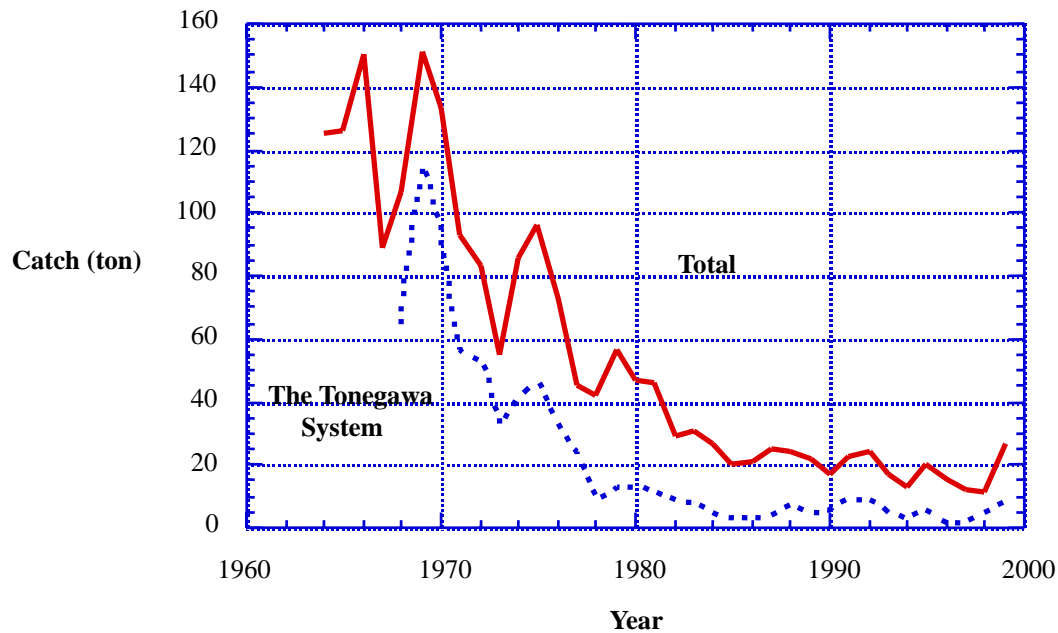

Figure 2. Annual catch of natural seeds for aquaculture in Japan. The annual catch is the sum of glass eel catch around coasts and seed catch (glass eel and juveniles up to one-year old) from the inland waterways.

Genetic Structure

The first obstacle to eel resource management is the lack of information on basic eel biology, such as spawning migration and behavior, larval ecology, and natural population dynamics and structure. This basic information must first be collected. We compared the frequency difference of each RAPD band to investigate the genetic structure of the Japanese eel from Taiwan (n=10) and Japan (south to north, n=25 each: Kagoshima, Ibaraki, and Miyagi). Glass eel were collected in Tung-Kang, Taiwan, on February 26, 1998, in Tanegashima,

Kagoshima, on January 28, 1998, and in Ibaraki and Miyagi, on February 28, 1998. The total DNA was extracted from each specimen by the regular phenol-chloroform method. RAPD primers used were Operon A1 to A20 and B1 to B7 (Operon Technologies, Alameda, CA, USA). Fifty nanograms of template DNA was in the 50μl PCR solution. A ready-to-use reaction mixture (PerfectShot™ Ex Taq, TaKaRa, Shiga, Japan) was used for PCR amplification. Reactions started at 94°C for four minutes and were amplified through 35 cycles at the following parameters: one minute at 94°C, one minute at 36°C, and two minutes at 72°C, followed by a final extension step at 72°C for five minutes. PCR products were separated on 0.9 and 1.4% agarose gels and visualized by ethidium bromide staining.

Thirteen 10-base random primers (A1, A4, A5, A8, A10, A11, A12, A16, A17, A18, A20, B1, and B5) produced scorable clear bands through PCR. Frequencies of most RAPD bands were similar among the four locations. The 1100bp band amplified by the primer A10 was absent in the Miyagi samples and present in the remaining populations at different frequencies (Table 1; 0.11-0.24). The difference between Miyagi and the other three sites was nearly significant (Mann-Whitney U test: p=0.061). Moreover, the 1000bp band amplified by the primer B1 was present in Miyagi at the low frequency (0.08), and the other three sites did not express the band. Although the frequency of the 1500bp band amplified by the primer A11 was 0.39 in Kagoshima, the remaining sites had higher frequencies (0.70-0.82).

Table 1. Frequency of A10 RAPD markers for Japanese eel samples from Japan and Taiwan.

Location	RAPD marker (bp)						
	1000	1100	1200	2020	3200	3300	3500
Miyagi (n=24)	0.92	0.00	0.67	0.96	0.33	0.25	0.08
Ibaraki (n=25)	1.00	0.16	0.68	0.92	0.28	0.20	0.08
Kagoshima (n=25)	0.96	0.24	0.76	1.00	0.40	0.52	0.20
Taiwan (n=9)	1.00	0.11	0.56	1.00	0.22	0.22	0.00

Discussion

Adult eel and glass eel catches in Japan have declined with some fluctuations for the last 40 years. However, Japanese consumers are not aware of the problem because they can buy European eel cultured in China at reasonable prices year around. Although the causes for the reduction in the number of glass eel are not clear, there are four possibilities: 1) habitat loss due to construction of dams, 2) pollution in estuaries and rivers, 3) overfishing of glass eel, and 4) global environmental changes. Unfortunately, none of these hypotheses has been tested.

It is difficult to speculate which hypothesis is correct because the population dynamics of eel in the entire life cycle is unknown. The life history of the Japanese eel is only partially understood. Spawning sites of the Japanese eel appear to be west of the Mariana Islands in the North Pacific (Tsukamoto, 1992), and eel travel several thousand kilometers to spawn. The leptocephalous larvae travel back to freshwater habitats in Taiwan, mainland China, Korea, and Japan. A recent study showed that some larvae seemed to stay in the ocean to grow (Tsukamoto *et al.*, 1998). The spawning migration and population dynamics and structure of the Japanese eel

in nature are not well understood.

A previous allozyme study indicated that there were allelic clines at two loci in *Anguilla japonica* (Chan *et al.*, 1997). However, mtDNA studies of *A. japonica* did not reveal genetic structuring (Sang *et al.*, 1994; Ishikawa *et al.*, 2001). Although our results did not show significant genetic differentiation, additional genetic studies with a large sample size (ca. 100 individuals per location) are necessary to determine the genetic structure of the Japanese eel. Prolonged spawning periods predicted from the presence of leptocephali (Cheng and Tzeng, 1996; Tsukamoto *et al.*, 1998) may cause temporal segregation among populations.

Cumulative knowledge of the life history and ecology of the target species is the key to success for the resource management and stock enhancement efforts. The research will help not only artificial propagation but also resource conservation. Efforts to protect wild eel resources need to continue and we should not be totally dependent on the future success of artificial propagation.

Acknowledgements

Literature Cited

Chan, I. K. K., D. K. O. Chan, S. C. Lee, and K. Tsukamoto. 1997. Genetic variability of the Japanese eel *Anguilla japonica* (Temminck & Schlegel) related to latitude. *Ecol. Freshwater Fish.* 6:45-49.

Cheng, P. W. and W. N. Tzeng. 1996. Timing of metamorphosis and estuarine arrival across the dispersal range of the Japanese eel *Anguilla japonica*. *Mar. Ecol. Prog. Ser.* 131:87-96.

Ishikawa, S., J. Aoyama, K. Tsukamoto, and M. Nishida. 2001. Population structure of the Japanese eel *Anguilla japonica* as examined by mitochondrial DNA sequencing. *Fish. Sci.* 67:246-253.

Kasebayashi, T. 1960. On the supply of the seed eel in the Tone River system. Research Report of the Fisheries Office of Kasumigaura and Kitaura in Ibaraki Prefecture 5:1-11 (in Japanese).

Matsui, I. 1952. Studies on the morphology, ecology and pond-culture of the Japanese eel (*Anguilla japonica* Temminck & Schlegel). *J. Shimonoseki Coll. Fish.* 2:1-257.

Sang, T.-K., H.-Y. Chang, C.-T. Chen, and C.-F. Hui. 1994. Population structure of the Japanese eel, *Anguilla japonica*. *Mol. Biol. Evol*. 11:250-260.

Tanaka, H., H. Kagawa, and H. Ohta . 2000. The first success in production of leptocephali of Japanese eel (*Anguilla japonica*) in captivity. Major Research Achievements of National Research Institute of Aquaculture in 1999. 4-6.

Tsukamoto, K. 1992. Discovery of spawning area for the Japanese eel. *Nature* 356:789-791.

Tsukamoto, K., T.-W. Lee, and N. Mochioka. 1998. Synchronized spawning of *Anguilla japonica* inferred from daily otolith increments in leptocephali. *Ichthyol. Res*. 45:187-193.

Tsukamoto, K., I. Nakai, and F.-W. Tesch. 1998. Do all freshwater eels migrate? *Nature* 396:635-636.

Comparative Diets and Growth of Two Scombrid Larvae, Chub Mackerel *Scomber japonicus* and Japanese Spanish Mackerel *Scomberomorus niphonius*, in the Central Seto Inland Sea, Japan

Jun Shoji
Masaru Tanaka
Laboratory of Marine Stock-Enhancement Biology
Division of Applied Biosciences, Graduate School of Agriculture
Kyoto University
Kitashirakawa, Kyoto 606-8502
JAPAN
Email: shog@kais.kyoto-u.ac.jp

Tsutomu Maehara
Toyo Branch
Ehime Prefecture Chuyo Fisheries Experimental Station
Kawarazu, Toyo, Ehime 799-1303
JAPAN

Abstract

Occurrence, distribution, feeding habits and growth during the early life stages of two scombrids, chub mackerel *Scomber japonicus* and Japanese Spanish mackerel *Scomberomorus niphonius,* were studied to elucidate their early life histories. Larvae and early juveniles were collected by larva-net tows and commercial boat seine fisheries in the central Seto Inland Sea, Japan in 1995 and 1996. Larvae of the two mackerels were caught in both May and June, with peak abundance in late May for chub mackerel and in early June for Japanese Spanish mackerel. Analysis of gut contents revealed that the feeding habits of chub mackerel larvae were complex compared to those of Japanese Spanish mackerel larvae. Most of the gut contents of chub mackerel larvae were composed of Copepoda, Appendicularia and other invertebrate plankters. Fish larvae were found in the guts of chub mackerel larvae larger than 5 mm SL and its occurrence rate increased as growth proceeded. On the other hand, Japanese Spanish mackerel larvae fed almost exclusively on fish larvae from the first feeding. The growth rate during larval and early juvenile stages of Japanese Spanish mackerel (1.47 mm/day), assessed by otolith microstructure, was higher than that of chub mackerel (0.71 mm/day). Japanese Spanish mackerel larvae could be considered to have a unique food habit which results in extremely rapid growth and reduced vulnerability to predation in the early life stages.

Two scombrid fishes, chub mackerel *Scomber japonicus* and Japanese Spanish mackerel *Scomberomorus niphonius*, are commonly found along the coastal waters of Japan. Spawning migration of the two species from oceanic waters (the northwestern Pacific) into the Seto Inland Sea (Fig. 1) occurs during the spring (Mito, 1965; Kishida and Aida, 1989). Although chub mackerel is an important fisheries resource, there is no information on its survival or recruitment in the Seto

Inland Sea. Comprehensive biological studies on the early life history of chub mackerel were conducted mainly in the oceanic waters around Japan, such as the Kuroshio and the Tsushima Current regions (Watanabe, 1970; Uchida *et al.,* 1958). Feeding habits of larval chub mackerel were investigated in the southeastern Pacific (Lipskaya, 1982) and southeastern waters of Japan (Yokota *et al.*, 1961). Japanese Spanish mackerel is one of the most important fisheries resources in the Seto Inland Sea, where total catch exceeded 6,000 t in the mid 1980s. The stock biomass, however, has been decreasing and is less than one-twentieth of that in the 1980s. In the central Seto Inland Sea, one of the main spawning grounds of

Figure 1. Map of the Hiuchi-nada, central waters of the Seto Inland Sea, showing the sampling area where ichthyoplankton was collected in 1995 and 1996. Closed squares indicate stations sampled for seasonal occurrence and closed circles stations sampled for distribution of chub and Japanese Spanish mackerel larvae, respectively. Shaded areas indicate areas where the boat seine fishery operated.

Japanese Spanish mackerel, the total catch has decreased to 74 t in 1997, while that of chub mackerel has increased to ca. 500 t in the 1990s (Fig. 2).

In order to stabilize the catch and to establish more effective fisheries management, it is necessary to understand the early life histories and recruitment processes of these species. Here, we describe the early life histories of chub mackerel and Japanese Spanish mackerel in the Seto Inland Sea with emphasis on larval piscivory.

Methods and Materials Three sampling regimes were carried out in the Hiuchi-nada, central waters of the Seto Inland Sea, Japan (Fig. 1). One set of surveys was conducted on the larval occurrence of chub mackerel and Japanese Spanish mackerel (cruise SO) two to three times per month, from March to June 1995 and April to June 1996, on the RV Hiuchi (4.9 t) and RV Yuri (4.9 t) of Ehime Prefecture Chuyo Fisheries Experimental Station. Ten-minute middle layer tows with a conical larva-net (mouth diameter 1.3 m, mesh aperture 1.0 mm) were made to collect these species.
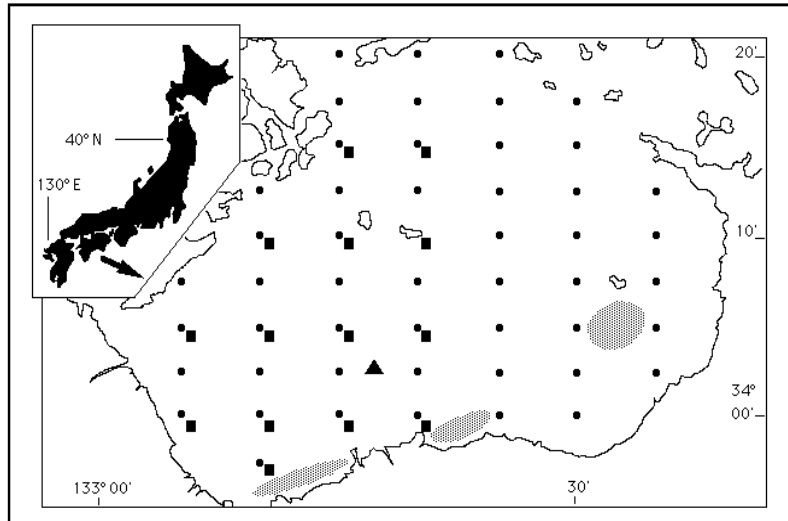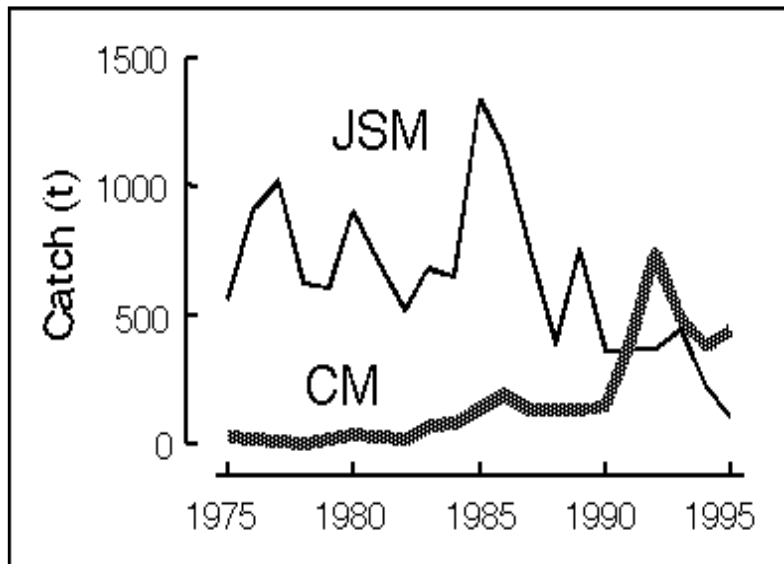

Figure 2. Trends of annual catch of chub mackerel (CM) and Japanese Spanish mackerel (JSM) in the Hiuchi-nada, central waters of the Seto Inland Sea, 1975 to 1995.

Another set of surveys was performed to study the horizontal distribution of mackerel larvae (cruise SH) during cruises of the RV Shirauji-maru (138 t) of National Research Institute of Fisheries and Environment of the Inland Sea on 27-30 May and 18-21 June 1996. Oblique hauls were made from the surface to 5 m above the sea floor using a bongo net (mouth diameter 0.7 m, mesh aperture 0.315 mm) at 50 stations.

Advanced-stage larvae and early juveniles of chub and Japanese Spanish mackerels were then collected in June and July, 1995 and 1996 from catches by a boat seine fishery, which operates primarily to fish Japanese anchovy *Engraulis japonicus* larvae and juveniles in the southern part of the survey area.

In cruises SO and SH, water temperature profiles were measured with STD. The volumes of seawater filtered by the nets were measured by a flowmeter mounted on the mouths of the nets and the ichthyoplankton catch from the horizontal and oblique hauls were converted to catch per $1000/m^3$ and $100/m^2$, respectively. Ichthyoplankton samples were preserved in 10% formalin and a subsample of chub and Japanese Spanish mackerel larvae was preserved in 90% ethanol within 24 hours after 10% formalin fixation for otolith analysis. The standard lengths (SL) and upper jaw lengths (UJL) of 160 chub and 130 Japanese Spanish mackerel larvae were measured and mouth diameters (MD) were calculated by the equation, $MD=UJL^{0.5}$ according to Shirota (1970).

All of the samplings during cruises SO and SH and the boat seine fishery were conducted in the daytime (8:00 a.m. to 5:00 p.m.). A total of 420 chub and 479 Japanese Spanish mackerel larvae were analyzed for stomach contents.

Growth in the early life stages of chub and Japanese Spanish mackerel larvae were estimated from otolith increments according to validations for each species. Daily otolith rings begin to form at hatching in Japanese Spanish mackerel (Shoji *et al.*, 1999) and at yolk exhaustion in chub mackerel (Brothers *et al.*, 1983). Since chub mackerel larvae initiate exogenous feeding at 48 hours after hatching under 19°C (Watanabe ,1970), otolith ages were corrected by adding two days to the total counts. A total of 80 chub and 105 Japanese Spanish mackerel larvae and early juveniles collected during the cruises and the boat seine fishery sampling was examined for growth analysis. Right-side sagittal otoliths of larvae were removed under a dissecting microscope and otolith increments were counted under a compound microscope with a video monitor.

Separation of larval chub mackerel *Scomber japonicus* from its congener, spotted mackerel *Scomber australasicus* is difficult (Ozawa, 1988). The difference in pigmentation of larvae between the two species may not always be definitive and the early larvae we collected in the Seto Inland Sea might include both *Scomber* species. The spotted mackerel, however, spawn in more oceanic waters of southwestern Japan (Tanoue, 1966). Therefore, in this study, we considered all of the *Scomber* larvae we collected during the cruises to be chub mackerel *Scomber japonicus* that originated in the Seto Inland Sea.

Results

Occurrence and Distribution of Larvae

In cruise SO, 949 chub and 248 Japanese Spanish mackerel larvae were collected. Seasonal variations in density ($1000/m^3$) of the larvae in 1995 and 1996 are shown in (Fig. 3). Both species were abundant from late May to early June, when the mean surface water temperature in late May was 18.0°C in 1995 and 18.3°C in 1996, and decreased after early June when the temperature averaged 18.9°C and 19.1°C, respectively. The seasonal occurrence of chub mackerel larvae was similar to that of Japanese Spanish mackerel larvae with a 10-day shift separating peak occurrence of the two species.

In cruise SH, 791 chub (2.3-10.4 mm SL) and 118 Japanese Spanish (3.6-10.8 mm SL) mackerel larvae were collected. On 27-30 May, which almost corresponds with the peak abundance of the two mackerels, the densities of chub mackerel larvae was higher at the central, southern and eastern stations (Fig. 4). Japanese Spanish mackerel larvae showed almost the same pattern of horizontal distribution as that of chub mackerel larvae, being abundant in the central and southern stations. On 18-21 June the two species were collected mainly at the central and southern stations, but less abundantly.

Figure 3. Seasonal variation of mean surface water temperature (WT) and mean density (number $1000/m^3$) of chub mackerel (CM) and Japanese Spanish mackerel (JSM) larvae obtained from the horizontal hauls (cruise SO) in the Hiuchi-nada in 1995 and 1996. E, M, and L indicate early, middle, and last 10 day periods of a month, respectively.
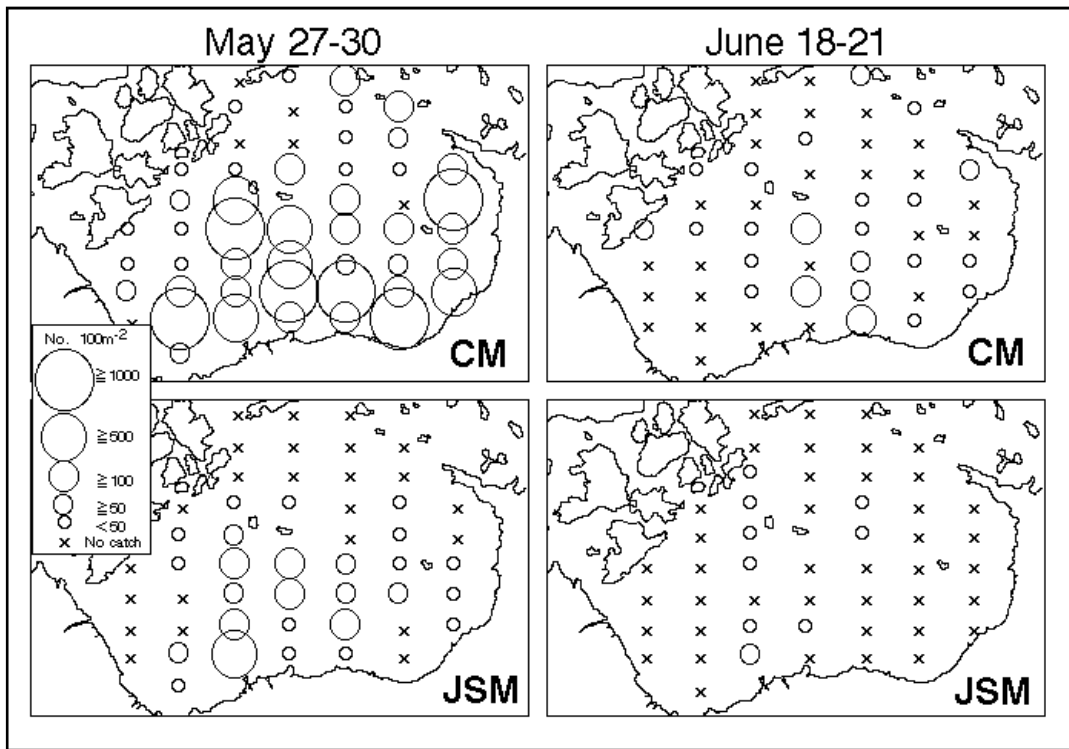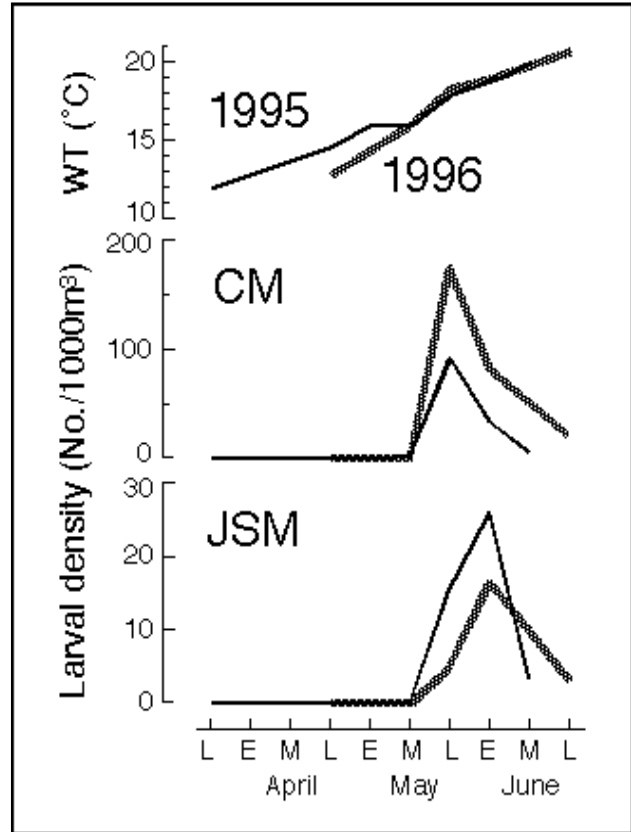

Figure 4. Distribution of chub mackerel (top: CM) and Japanese Spanish mackerel (bottom: JSM) larvae obtained from the oblique hauls (cruise SH) in the Hiuchi-nada on 27-30 May and 18-21 June in 1996.

<u>Gut Contents</u>

Analysis of gut contents revealed distinctive food habits between the two mackerel larvae (Table 1). Appendicularia, Copepoda and fish larvae were dominant taxa in the guts of chub mackerel larvae, occupying 34.0%, 30.8%, and 23.8% in number, respectively. Other invertebrate plankton taxa, such as invertebrate egg, Cladocera, Mysidacea and Decapoda occupied 0.5% to 3.2%. On the other hand, Japanese Spanish mackerel larvae fed almost exclusively on fish larvae despite the low numbers of invertebrate plankton observed. The dominant fish taxa eaten by chub and Japanese Spanish mackerel were Clupeiformes, which occupied 76.3% and 93.7%, respectively, of the identified fish in their stomachs.

Table 1. Feeding incidence and composition of stomach contents in terms of number of food items of chub mackerel (C) and Japanese Spanish mackerel (JSM) larvae.

	CM		**JSM**	
Size range (SL mm)	2.3-17.2		3.7-18.5	
No. of fish examined	420		479	
No. of fish feeding	358		411	
Guts with food (%)	85.2		85.8	
Contents	N	%	N	%
Invertebrate egg	14	2.1	1	0.2
Cladocera	3	0.5		
Copepoda	202	30.8	4	0.9
Mysidacea	21	3.2		
Decapoda	3	0.5		
Appendicularia	223	34.0		
Fish larva	156	23.8	386	90.8
Unidentified	34	5.2	34	8.0

The percentage occurrence of guts containing fish larvae is shown by size range in the two mackerel larvae (Fig. 5). None were found in the guts of chub mackerel larvae less than 5 mm SL. This percentage increased with chub mackerel larvae growth. Japanese Spanish mackerel larvae exhibited almost complete piscivory regardless of the larval size.

Mouth Size

Development of a large mouth, which may improve feeding efficiency, seems to affect the timing of the onset of piscivory in marine fish larvae. A previous study on the relationship between mouth size and prey size during the early life stages of chub mackerel revealed that the percentage of feeding success decreased as relative prey size (ratio of prey width to mouth width) increased (Hunter and Kimbrell, 1980). For piscivorous larval fish, mouth gape is considered an important factor in capturing piscine prey, which have advanced swimming ability, compared to invertebrate plankton prey (Hunter, 1981). Mouth diameter, calculated using UJL, of the two mackerels larvae increased as the larval growth progressed (Fig. 6). Mouth diameter of Japanese Spanish mackerel larvae in the first feeding stage was about 1 mm, twice that of chub mackerel larvae. The relationship between standard length (L, mm) and mouth diameter (D, mm) was expressed by the following equation:

Figure 5. Percentage of chub mackerel (CM) and Japanese Spanish mackerel (JSM) larval guts with fish larvae. Each 1 mm size class includes more than 20 observations

chub mackerel: $D=0.218*L-0.241$ ($n=160$, $r^2=0.97$)
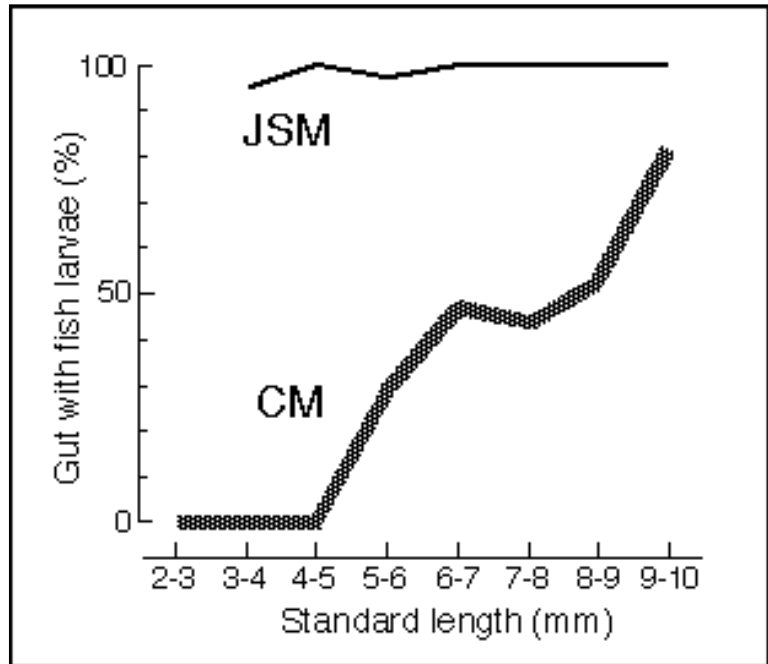Japanese Spanish mackerel: $D=0.487*L-1.431$ ($n=130$, $r^2=0.92$)

Figure 6. Relationship between mouth diameter (D. mm) and standard length (l. mm) of chub mackerel (CM) and Japanese Spanish mackerel (JSM) larvae.
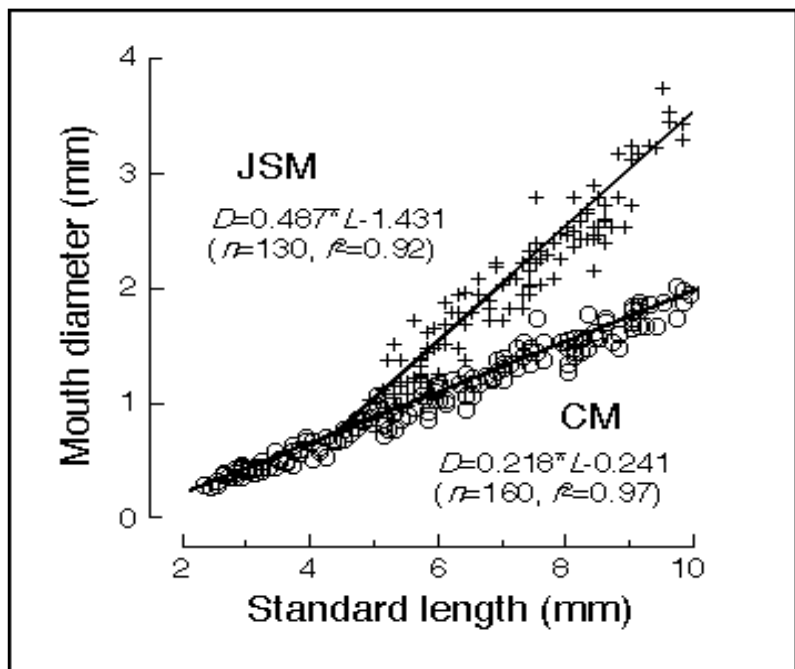
Figure 7. Relationship between standard length (*L.* mm) and estimated age in days (*d*) of chub mackerel (CM) and Japanese Spanish mackerel (JSM) larvae and early juveniles.

Growth

The relationship between standard length (*L*, mm) and age (*d, days*) during the larval and early juvenile stages was estimated from the otolith increments (Fig. 7) and expressed by the following exponential equations:
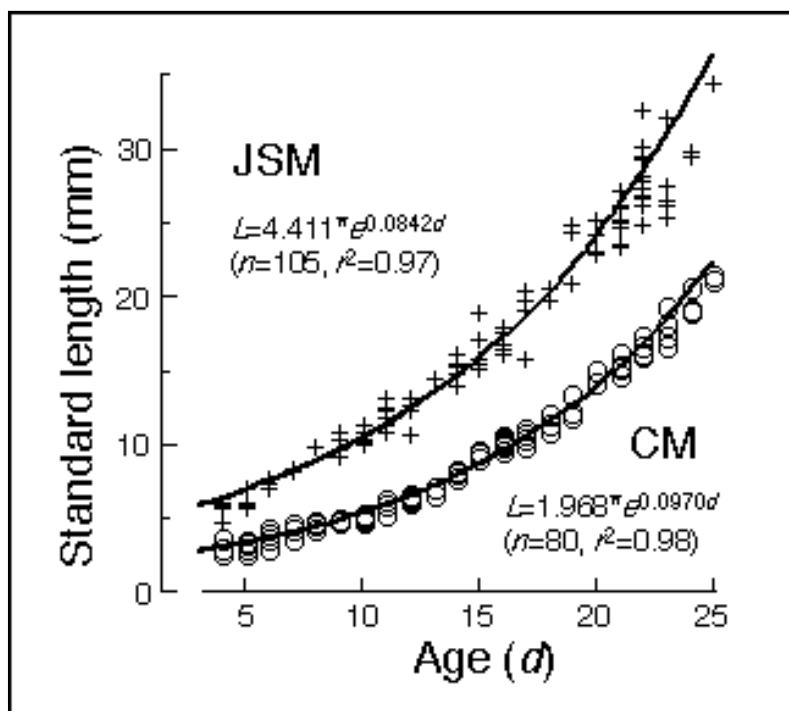
chub mackerel:	$L=1.968*e^{0.0970d}$ ($n=80$, $r^2=0.98$)
Japanese Spanish mackerel:	$L = 4.411*e^{0.0842d}$ ($n=105$, $r^2=0.97$)

The two species had similar specific growth rates, from 8 to 10 % of standard length per day. The predicted absolute growth rate after first feeding varied between developmental stages and species (Table 2). Timing of first feeding of chub and Japanese Spanish mackerel larvae were considered as 2 and 5 days after hatching, respectively (Watanabe 1970; Shoji *et al.* 2001). The absolute growth rate of Japanese Spanish mackerel increased from approximately 0.7 mm/day in the post-first-feeding stage to approximately 1.5 mm/day in the early juvenile stage.

Table 2. Predicted absolute growth rates (mm/day) as a function of age from first feeding of chub mackerel(CM) and Japanese Spanish mackerel (JSM) larvae and juveniles.

Age (day)	CM	JSM
5	0.298	0.704
10	0.391	0.888
15	0.523	1.136
20	0.712	1.474

Discussion

Chub and Japanese Spanish mackerel larvae appear to have similar patterns of temporal and spatial distribution in the central Seto Inland Sea. They showed a similar pattern of seasonal occurrence from May to June in 1995 and 1996 (Fig. 3). In both species, seasonal peaks in larval densities were observed in late May to early June, suggesting a short spawning period. Larval distribution patterns were also similar. In late May 1996, during the seasonal peak of occurrence in both, they were most abundant at stations in the central and southern areas of the Hiuchi-nada (Fig. 4). The timing of spawning migrations into the Inland Sea and the seasonal peak of larval abundance of chub and Japanese Spanish mackerels were well matched with the seasonal peak of ichthyoplankton prey abundance (Mito, 1964).

In the Seto Inland Sea, chub mackerel larvae initially fed on invertebrate plankton, primarily Appendicularia and Copepoda (Fig. 5, Table 1). These animals were also dominant prey of the chub mackerel in the southwestern waters of Japan (Yokota *et al.,*1961) and the southeastern Pacific (Lipskaya, 1982) waters of Japan. Fish appeared in the diet of chub mackerel larvae at the 5-6 mm SL size class and became more common as larval growth proceeded (Fig. 5). At 5-6 mm SL, the mouth diameter of chub mackerel larvae reached that of Japanese Spanish mackerel larvae at the first feeding (Fig. 6). Jaw and pharyngeal teeth (Kohno *et al*., 1984) and digestive structures (Tanaka *et al*., 1996) begin to develop between 5.0 to 7.0 mm SL in chub mackerel. These morphological developments are considered to be associated with the onset of piscivory.

Compared to our observations in the Seto Inland Sea, the onset of piscivory of chub mackerel larvae seems to be later in oceanic waters, such as southwestern Japan (Yokota *et al*., 1961), where chub mackerel larvae smaller than 8 mm fed only on invertebrate plankters. These results suggest that chub mackerel larvae can use flexibly two feeding tactics, planktivory and piscivory, depending on food availability in the water. Spawning migrations of many fishes into the Inland Sea result in rapid increase in ichthyoplankton density from spring to summer (Mito, 1964; Shoji *et al.,*1999). Higher food availability in the Seto Inland Sea than in the oceanic waters may allow the earlier onset of piscivory of the chub mackerel larvae observed in this study.

Piscivory was observed in Japanese Spanish mackerel larvae from the first feeding stage. The intensity of piscivory did not fluctuate with larval size (Fig. 5). Other fish larvae contributed to the majority of the stomach contents, while invertebrate plankters were seldom observed (Table 1).

Scombrid larvae generally have large eyes and a mouth and exhibit an early shift from planktivory to piscivory relative to other marine fish larvae (Hunter 1981). Some tuna larvae, such as bluefin tuna *Thunnus thynnus* and skipjack tuna *Katsuwonus pelamis,* have large eyes and mouth gapes (Shirota, 1978; Uotani *et al*., 1990) and exhibit piscivory in their early life stages (Nishikawa, 1975; Uotani *et al*., 1981; Young and Davis, 1990). In these species, however, piscivory appears to develop at a more advanced stage. We speculate that there are other factors affecting the onset of piscivory in scombrid larvae. First, size at initial feeding should be important because larger size enables capture of relatively large prey. Japanese Spanish mackerel larvae hatch from large eggs (1.35 to 1.85 mm in diameter) and exhaust their yolks at 6.0 mm SL (Xue-shen *et al*., 1966), while egg size (less than 1.2 mm; Mito, 1961) and larval size at yolk exhaustion (less than 4 mm; Yabe *et al*., 1966; Ueyanagi *et al*., 1974) are smaller in these tunas. Secondly, development of the digestive system also seems to be related to the early onset of piscivory. Tanaka *et al*. (1996) showed precocious development of an adult-type digestive system with a functional stomach and pyloric caeca of laboratory-reared Japanese Spanish mackerel larvae. Finally, an instinctive factor seems to be important, where Japanese Spanish mackerel larvae began to cannibalize when supplied with

only invertebrate plankton prey under rearing conditions (Fukunaga *et al.*, 1982; Shoji and Tanaka, 2001).

Our study supports the previous reports of higher growth rates during early life stages of members of the genus *Scomberomorus* than that of *Scomber* species. The absolute growth rate of the chub mackerel obtained from this study (0.712 mm/day, up to 20 days after first feeding, Table 2) was similar to that of Atlantic mackerel from the middle Atlantic Bight (Kendall and Gordon, 1981). In laboratory experiments, Hunter and Kimbrell (1980) observed that rapid increase in prey size was required to support rapid growth of chub mackerel larvae. In this study, absolute growth of sea-caught chub mackerel increased from 0.298 mm/day (post-first-feeding stage) to 0.712 mm/day (later larval and early juvenile stages). The increase in growth rate of chub mackerel observed in this study may result from the feeding shift to piscivory indicated by the analysis on the wild larvae (Fig. 5).

Higher growth rates were observed in king mackerel *Scomberomorus cavalla* (1.31 mm/day) and Spanish mackerel *Scomberomorus maculatus* (0.82 mm/day) larvae from the Gulf of Mexico and U. S. South Atlantic Bight (DeVries *et al.*, 1990). These rates were close to the absolute growth rate of the Japanese Spanish mackerel larvae and early juveniles up to 10 and 15 days after first feeding (0.888 and 1.136 mm/day, respectively, Table 2). The higher growth rates observed in *Scomberomorus* larvae are probably related to the specialized food habits at early onset of piscivory (DeVries *et al.*, 1990; Finucane *et al.*, 1990; Shoji *et al.*, 1997). Compared with the chub mackerel's relatively flexible feeding habits, Japanese Spanish mackerel larvae seem to have evolved a more specialized strategy of complete piscivory from the first feeding stage. Growth and survival of Japanese Spanish mackerel larvae may be highly dependent on piscine prey abundance relative to chub mackerel and other planktivorous larvae.

Acknowledgments

Literature Cited

Brothers, E. B., E. D. Prince, and D. W. Lee. 1983. Age and growth of young-of-the-year bluefin tuna, *Thunnus thynnus*, from otolith microstructure. NOAA Tech. Rep. NMFS 8:49-59.

DeVries, D. A., C. B. Grimes, K. L. Lang, and D. B. White. 1990. Age and growth of king and Spanish mackerel larvae and juveniles from the Gulf of Mexico and U.S. South Atlantic Bight. *Env. Biol. Fish*. 29:135-143.

Finucane, J. H., C. B. Grimes, and S. P. Naughton. 1990. Diets of young king and Spanish mackerel off the southeast United States. *Northeast Gulf Sci.* 11:145-153.

Fukunaga, T., N. Ishibashi, and N. Mitsuhashi. 1982. Artificial fertilization and seedling propagation of Spanish mackerel. *Saibai Giken* 11:29-48 (In Japanese).

Hunter, J. R. 1981. Feeding ecology and predation of marine fish larvae. pp. 33-77. <u>In</u>: Marine Fish Larvae. Lasker, R. (ed.), Univ. Washington Press, Seattle.

Hunter, J. R. and C. A. Kimbrell. 1980. Early life history of Pacific mackerel, *Scomber japonicus*. *Fish. Bull*. 78:89-101.

Kendall, A. W. and D. Gordon. 1981. Growth rate of Atlantic mackerel (*Scomber scombrus*) larvae in the Middle Atlantic Bight. *Rapp. P. -v. Reun. Cons. int. Explor. Mer.* 178:337-341.

Kishida, T. and K. Aida. 1989. Maturation and spawning of Japanese Spanish mackerel in the central and western Seto Inland Sea. *Nippon Suisan Gakkaishi* 55:2065-2074 (In Japanese with English abstract).

Kohno, H., M. Shimizu, and Y. Nose. 1984. Morphological aspects of the development of swimming and feeding functions in larval *Scomber japonicus*. *Nippon Suisan Gakkaishi* 50:1125-1137.

Lipskaya, N. Y. 1982. The feeding of larvae of the chub mackerel, *Scomber japonicus* (Scombridae), from the southeastern Pacific. *J. Ichthyol*. 22:97-104.

Mito, S. 1961. Pelagic eggs from Japanese waters-II. Lamprida, Zeida, Mugilina, Scombrina, Carangina and Stromateina. *Sci. Bull. Fac. Agr. Kyushu Univ.* 18:451-466 (In Japanese with English abstract).

Mito, S. 1964**.** Studies on the fish eggs and larvae found in the Seto Inland Sea-I. Abundance and distribution. *Rep. Naikai Reg. Fish. Res. Lab.* (Pub. Ser. C) 2:1-24 (In Japanese with English abstract).

Mito, S. 1965**.** Studies on the fish eggs and larvae found in the Seto Inland Sea-II. A list of species with some morphological and ecological notes. *Rep. Naikai Reg. Fish. Res. Lab*. (Pub. Ser. C) 4:1-17 (In Japanese with English abstract).

Nishikawa, Y. 1975. Feeding of larval and juvenile skipjack tuna in relation to the development of their stomachs. *Bull. Far Seas Fish. Res. Lab*. 12:221-231 (In Japanese with English abstract).

Ozawa, T. 1988. *Scomber australasicus* Cuvier, pp. 611-613. <u>In</u>: An atlas on the early stage fishes in Japan. M. Okiyama (ed.), Tokai Univ. Press, Tokyo (In Japanese).

Shirota, A. 1970. Studies on the mouth size of fish larvae. *Nippon Suisan Gakkaishi* 36:353-368 (In Japanese with English abstract).

Shirota, A. 1978. Studies on the mouth size of fish larvae-II. Specific characteristics of the upper jaw length. *Nippon Suisan Gakkaishi* 44:1171-1177 (In Japanese with English abstract).

Shoji, J., T. Kishida, and M. Tanaka. 1997. Piscivorous habits of Spanish mackerel larvae in the Seto Inland Sea. *Fish. Sci*. 63:388-392.

Shoji, J., T. Maehara, and M. Tanaka. 1999. Short-term occurrence and rapid growth of Spanish mackerel larvae in the central waters of the Seto Inland Sea, Japan. *Fish. Sci.* 65:68-72.

Shoji, J., T. Maehara, M. Aoyama, H. Fujimoto, A. Iwamoto, and M. Tanaka. 2001. Daily ration of Japanese Spanish mackerel *Scomberomorus niphonius* larvae. *Fish. Sci*. 67:238-245.

Shoji, J. and M. Tanaka. 2001. Strong piscivory of Japanese Spanish mackerel larvae from their first feeding. *J. Fish. Biol*. 59:1862-1865.

Tanaka, M., T. Kaji, Y. Nakamura, and Y. Takahashi. 1996**.** Developmental strategy of scombrid larvae: High growth potential related to food habits and precocious digestive system development. pp. 125-139. <u>In</u>: Survival Strategies in Early Life Stages of Marine Resources. Watanabe, Y., Y. Yamashita and Y. Oozeki (ed.), A. A. Balkema, Rotterdam.

Tanoue, T. 1966. Studies on the seasonal migration and reproduction of the spotted mackerel, *Pneumatophorus tapeinocephalus* (Bleeker). *Mem. Fac. Fish. Kagoshima Univ.* 15:91-175.

Uchida, K. 1958. *Scomber japonicus* (Houttuyn). pp. 47-49. <u>In</u>: Studies on the Eggs, Larvae and Juveniles of Japanese Fishes. Uchida, K., S. Imai, S. Mito, S. Fujita, M. Ueno, Y. Shojima, T. Senta, M. Tafuku, and Y. Dotsu (ed.), Series 1, *Second Lab. Fish. Biol. Dep. Fish. Fac. Agric. Kyushu Univ.*, Fukuoka (In Japanese with English abstract).

Ueyanagi, S., Y. Nishikawa, and T. Matsuoka. 1974. Artificial fertilization and larval development of skipjack tuna, *Katsuwonus pelamis*. *Bull. Far Seas Fish. Res. Lab.* 10:179-188 (In Japanese with English abstract).

Uotani, I., K. Matsuzaki, Y. Makino, K. Noda, O. Imamura, and M. Horikawa. 1981. Food habits of larvae of tunas and their related species in the area northwest of Australia. *Nippon Suisan Gakkaishi* 47:1165-1172 (In Japanese with English abstract).

Watanabe, T. 1970. Morphology and ecology of early stages of life in Japanese common mackerel, *Scomber japonicus* Houttuyn, with special reference to fluctuation of population. *Bull. Tokai Reg. Fish. Res. Lab.* 62:1-283 (In Japanese with English abstract).

Xue-shen, S., H. Gui-fen, and C. Hsiao-wei. 1966. A description of the morphological characters of the eggs and larvae of the blue spotted mackerel *Scomberomorus niphonius* (Cuvier & Valenciennes). *Oceanol. Limnol. Sin* 8:1-12 (In Chinese with English abstract).

Yabe, H., S. Ueyanagi, and H. Watanabe. 1966**.** Studies on the early life history of bluefin tuna *Thunnus thynnus* and on the larva of the southern bluefin tuna *T. maccoyii*. *Rep. Nankai Reg. Fish. Res. Lab.* 23:95-129 (In Japanese with English abstract).

Yokota, T., M. Toriyama, F. Kanai, and S. Nomura. 1961. Studies on the feeding habit of fishes. *Bull. Nankai Reg. Fish. Res. Lab*. 14:1-234 (In Japanese with English abstract).

Young, J. W. and T. L. O. Davis. 1990. Feeding ecology of larvae of southern bluefin, albacore and skipjack tunas (Pisces: Scombridae) in the eastern Indian Ocean. *Mar. Ecol. Prog. Ser.* 61:17-29.

Evaluating Stock Enhancement Strategies:
A Multi-Disciplinary Approach

Theresa M. Bert
Robert H. McMichael, Jr.
Richard P. Cody
Ann B. Forstchen
William G. Halstead
Joe O'Hop
John M. Ransier
Michael D. Tringali
Brent L. Winner
Frank S. Kennedy
Florida Fish and Wildlife Conservation Commission
Florida Marine Research Institute
100 Eighth Avenue Southeast
St. Petersburg, Florida 33701
Email: T.M. Bert, theresa.bert@fwc.state.fl.us

Kenneth M. Leber
Carole L. Neidig
Mote Marine Laboratory
Center For Fisheries Enhancement
1600 Ken Thompson Parkway
Sarasota, FL 34236

Abstract

Stock enhancement, the supplementation of depleted wild fish and invertebrate stocks with individuals reared in aquaculture facilities or captured from other populations, is becoming an increasingly popular method of bolstering heavily fished populations. Although many different aspects of marine stock enhancement have been evaluated for several species of fish and invertebrates, a multidisciplinary approach is often not feasible for many programs. In addition, a systematic, coordinated, comprehensive, monitoring program is not commonly used to test whether stock enhancement efforts actually result in measurable increases in fishery output. In 1999, the Florida Marine Research Institute in St. Petersburg, Florida, and Mote Marine Laboratory in Sarasota, Florida, initiated a multiyear stock enhancement experiment to supplement the red drum (*Sciaenops ocellatus*) population in Tampa Bay, Florida, USA. The original experimental design for releasing aquacultured (hatchery-reared) red drum into Tampa Bay included the following variables: two riverine systems, several sections within each system, two times of the year for release of the fish, and three categories of red drum size-at-release. The ongoing monitoring effort involves the following general categories of activities: breeding and rearing the fish to the stage of growth at which they are designated to be released into the estuaries; developing and using a multigene genetic tag to determine parentage of the hatchery-reared fish and to distinguish those fish from wild fish for many applications; designing and conducting comprehensive, fisheries-dependent and fisheries-independent, field sampling and monitoring programs to obtain information on the survival and dispersal of the hatchery-reared fish after their release, the entry of those fish into the reproductive population, and the contribution of those fish to recreational fishery landings; informing fishermen of the stock enhancement program and soliciting their participation; and monitoring the health of the hatchery-reared fish before and after their release and of the wild fish in the recipient red drum population. Here, we describe the general methodologies and the intergroup coordination used by the research groups charged with developing and executing the Tampa Bay red drum stock enhancement experiment.

Introduction

Red drum (*Sciaenops ocellatus*) are among the marine species most important to shallow-water and nearshore sportfishers (anglers) in the southeastern USA. Because they are easily available to nearshore-marine and dockside recreational fishermen, they are highly sought in a directed fishery in Florida. Loss or degradation of seagrass habitat, coastal development and associated chronic pollution, and heavy fishing pressure have reduced the number of red drum to a fraction of their former numbers, resulting in severe regulations that limit harvest. In the mid-1980s, the National Marine Fisheries Service declared some red drum stocks to be overfished. Between 1985 and 1987, a series of increasingly restrictive rules governing the red drum fishery were written by the Florida Marine Fisheries Commission, culminating in a rule that indefinitely prohibited all commercial fishing for red drum. Despite these measures, by 1988, the stock appeared to have declined to approximately 5% of its unfished biomass, implying that red drum reproductive potential might be inadequate to sustain local populations (Murphy and Crabtree, 2001). To address this problem and other related issues, staff of the Florida Fish and Wildlife Conservation Commission's Florida Marine Research Institute (FMRI) developed the Stock Enhancement Research Facility (SERF) between 1985 and 1988. In 1999, a multiyear project was initiated to enhance the depleted red drum stock in Tampa Bay, Florida. This project consists of an experimental phase and production phase and is currently in the experimental phase.

The technology now exists to rear large numbers of juvenile red drum in captivity at SERF. However, little is known about when or where to release these fish into Florida's estuarine systems or about the size of red drum that should be released to maximize their survival and to clearly show an increase in adult spawning stocks or fishermen's catches. In the experimental phase of this ongoing stock enhancement project, the influences of location, season of release, and size of release on the short-term and long-term survival of aquacultured (hatchery-reared) red drum stocked into Tampa Bay are being tested.

Here, we describe the methodology for our multi-disciplinary approach to evaluate red drum stocking strategies directed toward enhancing the Tampa Bay red drum population. Through our monitoring effort, we will estimate the short- and long-term survival of the stocked fish, their contribution to the local red drum breeding stock, their contribution to the harvested population, and the long-term genetic impact on wild red drum populations.

Methodology

Upon careful examination of various strategies for releasing hatchery-reared red drum into Tampa Bay, we decided on the following protocol. We release the fish into two rivers within the Tampa Bay estuarine system: the Alafia and Little Manatee Rivers (Fig. 1., following page). Both of these rivers are highly productive nursery areas for wild red drum (Peters and McMichael, 1987). We release three size-classes of fish: Phase I (25-45 mm standard length [SL]), Phase II (65-110 mm SL), and Phase III (>135 mm SL).

We release fish at two times of the year: "in-season" (the timing of spawning of wild and hatchery broodstock is approximately the same; thus the size range of the stocked fish closely matches the size range of the same wild-fish cohort) or "out-of-season" (the timing of spawning of that hatchery broodstock is approximately six months after the time of the wild-stock spawning; thus, the size range of the stocked fish differs notably [usually significantly] from that of any wild cohort). We release the fish at different locations along the rivers; these locations are

defined using a grid system in which the rivers are stratified according to distances from their river mouths. The stratification reflects shifts in salinity and temperature regimes from estuarine to marine at the mouths of the rivers.

The complete monitoring program involves the staffs of five separate, but integrated, research programs at FMRI and the staff of the Center for Fisheries Enhancement at Mote Marine Laboratory (MML), a non-profit marine laboratory located in Sarasota, Florida. The FMRI Fisheries Stock Enhancement (FSE) staff breeds adult red drum broodstock, rears their offspring to the appropriate size for release, physically tags all Phase II and Phase III fish with coded-wire tags (CWTs), and participates in all releases in the Alafia and Little Manatee rivers. The FMRI Biochemical Genetics

Figure 1. Location of the Alafia and Little Manatee rivers in Tampa Bay, Florida, USA. Insert shows location of Florida in the southeastern USA.

Laboratory (BGL) staff uses a multigene genetic tag to estimate the proportion of hatchery-reared fish in the post-enhancement (admixed) population at various times after the release and at various distances from the release sites. The BGL staff uses a multigene genetic tag to estimate the proportion of hatchery-reared fish in the postenhancement (admixed) population at various times after the release of hatchery-reared fish and at various distances from the release sites, to determine the effective population sizes of the broodstock and the broods, to determine the uniqueness of Phase-I offspring genotypes compared to the genotypic composition of wild-population red drum in the same size cohort, to estimate the proportion of hatchery-reared fish in the Tampa Bay red drum population, and to monitor the long-term genetic impact of the stock enhancement effort on the genetic diversity of the wild red drum population. The FMRI Fishery Independent Monitoring (FIM) and MML staffs routinely and systematically collect red drum from the admixed population; determine the proportion of tagged (CWTs or ultrasound transponders) Phase II or Phase III fish in the size cohorts that could contain those fish; and deliver all fish in the size cohorts that could contain Phase I fish, as well as all unmarked fish in the size cohorts that could contain Phase II or Phase III fish, to the BGL staff for genetic identification. The FMRI Fisheries-Dependent Monitoring (FDM) staff routinely and systematically surveys recreational fishermen to monitor their effort versus catch of red drum, to examine the harvested red drum for presence of CWTs and to obtain tissue samples from all other harvested red drum for genetic identification as wild or hatchery-reared fish. The FMRI Aquatic Health Group (AHG) staff evaluates the health of all hatchery-reared red drum offspring prior to their release and routinely assesses the health status of hatchery-reared and wild red drum captured by the FIM staff in their post-enhancement surveys. The MML staff conducts an
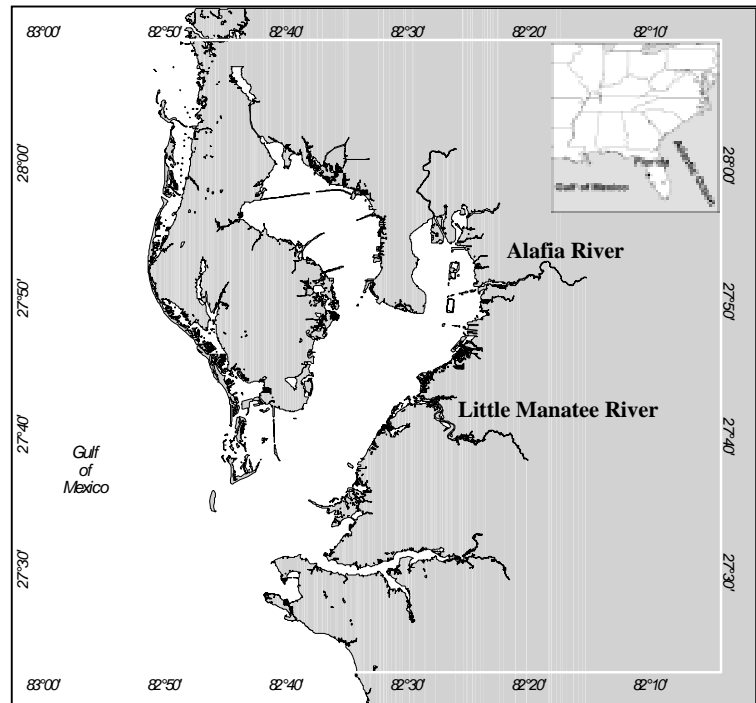
extensive advertising campaign geared toward angler awareness and participation in the program, collects fish samples from the Little Manatee River in a manner similar to that used by the FIM staff in the Alafia River, and delivers the appropriate samples to the BGL staff for genetic identification.

The path of a complete enhancement cycle is as follows. The FSE staff, with assistance from others at FMRI, captures wild, adult red drum for potential use as broodstock. The potential broodstock fish are tagged with Passive Integrated Transponder tags (American Veterinary Identification Device Company, Norco, California) and their multigene, genetic-tag genotypes are established by the BGL staff. Selected broodstock individuals are grouped into breeding aggregates and induced to spawn at SERF. Their offspring are reared at SERF to specific size-classes (Phases) and are released into specific sections of the Alafia River or Little Manatee River. Prior to release, the AHG staff evaluates the health of each brood and the FSE tags all Phase II and Phase III fish with CWTs. Both pre- and post-enhancement collections of red drum are obtained from selected locations in Tampa Bay during routine or directed field sampling efforts performed by the FIM and MML staffs. Fish that have CWTs are identified at the time of sampling. All other fish in cohorts that could contain hatchery-reared fish are delivered to the BGL staff for genetic identification. The FDM staff checks red drum harvested by recreational fishermen for the presence of CWTs and, with the permission of the anglers, obtains tissue samples from all fish without CWTs. These tissue samples are also delivered to the BGL staff for genetic identification. The BGL staff assays the fish from the FIM and MML post-enhancement sampling, the FDM recreational-fishermen surveys, and the MML angler-participation endeavor for the multigene genetic tag to ascertain with high probability the origin (hatchery-reared or wild) of these red drum collected from the admixed population.

BGL Genetic Identification Procedures

Although logically, broodstock spawning and offspring rearing are the initial steps in any stock enhancement project, we describe the work of the BGL staff first because the genetic identification component of this project is integrated into all other project components.

Central to the genetic monitoring program is a multigene genetic tag composed of a 419 nucleotide-base-pair (bp) region located in the mitochondrial DNA (mtDNA) control region plus nine nuclear-DNA microsatellite loci. In most animals, including red drum, mtDNA is transmitted uniparentally from mother to offspring (Wilson *et al.,* 1985; T. M. Bert and M. Tringali, unpublished data). Typically, the mtDNA control-region nucleotide sequence is highly variable among individuals in populations of marine fishes (Graves, 1998), including red drum (Seyoum *et al.*, 2000). Microsatellites are regions of nuclear DNA composed of sequential repeats of short nucleotide sequences that are typically 2-5 bp in length (Hillis *et al.*, 1996). Microsatellite DNA alleles are inherited from both parents. Allelic polymorphism in microsatellite DNA is measured as the variation in the number of these repeated units and is manifested in genetic assays as DNA fragments of different lengths. Levels of polymorphism and numbers of alleles at microsatellite loci are generally high within species and populations and are therefore useful for parentage analyses and as components of genetic tags.

The BGL staff obtains both the mtDNA and microsatellite genotypes of all female red drum and the microsatellite genotypes of all male red drum held at SERF for potential use as broodstock. In red drum mtDNA maternity studies, which involved >1000 offspring and 14 broodstock mothers, BGL staff found no instances where the mtDNA genetic-tag sequences of the mothers and their offspring differed (unpublished data), nor do they expect to find any in the

future, based on reported mutation rates for the mtDNA control region (Merilä *et al.*, 1997). Thus, because of the unique mode of inheritance of mtDNA, the mtDNA genotypes of the broods are known if the mtDNA genotypes of the female broodstock are known. Red drum from post-enhancement collections whose mtDNA genetic-tag sequences do not match those of any SERF female broodstock individual are highly unlikely to be stocked fish.

The mtDNA control-region sequence data are obtained by using standard Polymerase Chain Reaction (PCR) procedures (Saiki *et al.*, 1988) to amplify (make many copies of) the target mtDNA and an ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, CA) to obtain the mtDNA nucleotide sequences. The BGL staff also uses the PCR technique to amplify the microsatellite loci. They multiplex the PCR reactions (make many copies of the alleles from several different microsatellite loci simultaneously) and identify the genotypes using an ABI 310 Genetic Analyzer. Additional details of the laboratory procedures for obtaining the mtDNA control-region genetic-tag nucleotide sequences and the allelic patterns for several of the microsatellite DNA loci, as well as the levels of genetic variation in these DNA segments, are described in Seyoum *et al.* (2000) and Turner *et al.* (1998). The utility of these highly variable DNA segments for genetic tagging and the benefits of using multigene genetic tags in aquaculture are described in detail in Bert *et al.* (2001), Bert *et al.* (2002), and the references therein.

The compound genetic tag is used for a number of purposes in this complex stock-enhancement monitoring program (Bert and Tringali, in preparation). Here we describe use of the tag to distinguish hatchery-reared offspring from wild red drum that are in the same cohort in the post-enhancement red drum samples delivered to the BGL staff.

To determine the baseline level of genetic variation for both the mtDNA and microsatellite DNA components of the genetic tag, the BGL staff analyzed approximately 250 young-of-the-year (YOY) red drum each year from 1998 through 2000 from selected locations in Tampa Bay. To those data they added the genetic-tag genotypes of all fish captured for potential use as broodstock and then characterized the level of variation in this "library" of genetic-tag data. The genetic data library is used in several ways. One use is to estimate the frequency of the mtDNA genetic-tag genotype of each potential broodstock female using wild-population data as the basis for the frequency estimation and to classify each broodstock female based on that frequency. Each female having an mtDNA genotype not previously seen in a surveyed individual is classified as "unique." Each female possessing an mtDNA genotype previously encountered in one or two other fish is classified as "rare." Each female possessing an mtDNA genotype previously seen in three or more individuals is classified as "common."

Red drum captured by FIM, MML, or FDM staffs and provided to the BGL staff are first individually assayed for the mtDNA genetic-tag component. Any fish with an mtDNA sequence that matches that of a female broodstock individual is considered to be a "candidate" hatchery-reared fish and is subsequently analyzed for the microsatellite loci. Via parentage analyses, the results from the microsatellite analysis either support or do not support the SERF origin of that red drum. If the microsatellite DNA analysis does not exclude a SERF origin for an individual, the probability (based on the likelihood ratio) that the individual is actually a hatchery-reared fish is computed (Brenner, 1983). These ratios are based on the frequencies of the specific multilocus, microsatellite-DNA genotypes in the red drum analyzed to obtain the baseline data. Typically, these ratios are very small; thus the likelihood of correct positive identifications are very high ($> 99.999\%$).

FSE Broodstock Spawning, Offspring Rearing, and Release

Over the past fifteen years, the technology has been developed for spawning red drum in captivity by manipulating temperature and photoperiod and for rearing offspring in very large numbers in outdoor man-made ponds (Colura *et al.*, 1976; Arnold *et al.*, 1977; Roberts *et al.*, 1978). In general, adult red drum are collected sporadically throughout the year for use as broodstock. The BGL staff advises the FSE staff on which females to use to produce each brood based on the frequencies of the mtDNA genotypes of those females in the wild population. Their objective is to create broodstock groups that collectively have genetic-tag genotype compositions that distinguish the broods released for each experimental treatment from each other and from the wild population. After the genetic classifications of the potential broodstock females are obtained from the BGL staff, selected females are assigned to broodstock groups at the appropriate times and are placed into 16,000-l circular spawning tanks along with 3-4 males with ripe gonads. Depending on availability, 1-3 unique or rare females are used per broodstock group to produce fish that will be released in Phase I. Phase I red drum broods constitute about 80% of all released fish. There are no constraints on the genetic-tag genotypes or number of females used to produce fish that will be released in Phase II or Phase III. This is because a tenable, nongenetic system of physically tagging Phase I fish (e.g., CWT insertion or oxytetracycline marking of the otoliths) has not yet been developed and the amount of microsatellite DNA analysis required from the BGL staff is reduced. All hatchery-reared red drum that are released as Phase II or Phase III individuals are tagged with CWTs. The CWTs used by FSE are made of stainless steel and measure 1.00-mm long X 0.25-mm wide. Each wire contains a unique decimal code that identifies each fish as being of SERF origin, the broodstock group from which it was spawned, the location and date of release, and the mean size of the brood at the time of release. The genetic assays of Phase II and Phase III individuals not tagged with CWTs serve as a backup to the physical tagging and allow estimation of CWT loss or oversight.

To prepare a broodstock group for spawning, the fish are subjected to appropriate photothermal conditioning to induce gonadal maturation that culminates in spontaneous spawning. When the experimental design requires contemporaneous spawning from several broodstock groups (e.g., to synchronize the rearing of genetically distinct broods for multiple, simultaneous releases), spawning is induced hormonally if the females fail to spawn spontaneously. When hormone induction is necessary, FSE staff implant photothermally conditioned, gravid females with gonadotropin-releasing-hormone time-released pellets or inject them with human chorionic gonadotropin before placing them in tanks with sexually mature males in a prespawning condition. Spawning typically occurs within 30 hours of hormone administration.

After all spawning events, the eggs are harvested from spawning tanks using a 100-l egg collector that skims them from the surface of the water. The eggs are removed, counted volumetrically, and transported to an incubation system for acclimation and hatching. Approximately 60-66 hr after hatching, the larval red drum are transferred to 1-acre outdoor culture ponds. The fish are reared in the ponds until, collectively, they attain the appropriate mean size for their designated phase of release. During the entire rearing process, each brood is maintained separately from other broods. Each brood is also designated a specific site and approximate date for release. Immediately prior to harvesting, samples of fish are provided to the AHG staff for health evaluation and to an independent laboratory for health certification.
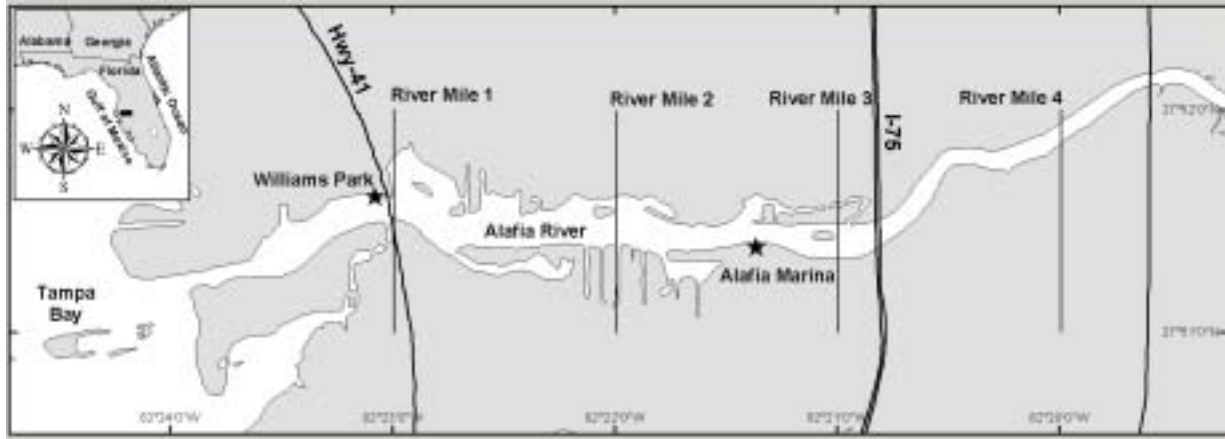
Figure 2a. Red drum (*Sciaenops ocellatus*) release and sampling domains. Insets show general location of each river in Florida. Release sites were designated as shorelines within each river section where suitable habitat was present. Alafia River; the four river miles delineate the four river grids used in the stock enhancement experiment.

When the red drum reach the appropriate size, the pond is drained to a depth of only a few centimeters and the fish are collected by net. Fish harvested in the Phase I size-class are immediately released. Fish harvested in the Phase II or Phase III size-classes are held in 16,000-liter, indoor, recirculating fiberglass tanks and observed for a recovery period of at least three days. Phase II and Phase III red drum are then tagged with CWTs, which are implanted vertically in the left adductor mandibularis with a Mark IV tagging machine (Northwest Marine Technology, Incorporated [NMT], Shaw Island, Washington). Immediately after being tagged, each red drum is scanned with a Quality Control Device (tube-detector) or Field Sampling Device (V-detector [NMT]) to verify the presence of the tag. The fish are again moved to the holding tanks for another recovery period of at least three days**.** They are then transported to the release site in a live-fish hauler (mobile tank) at a density of no more than 20 g of fish/l of seawater.

FIM and MML Collecting and Validation of Fish Origin

As their component of the stock enhancement experiment, the FIM staff is charged with co-operating with FSE staff in the release of the hatchery-reared red drum into the Alafia River, monitoring the relative abundance of red drum juveniles in the size cohorts that could contain hatchery-reared red drum, examining Phase II and Phase III fish for the presence of CWTs, and providing the BGL staff with tissue samples (usually fin clips) of all red drum that are not tagged but are within the size ranges in which the stocked red drum might occur.

The hatchery-reared red drum destined for release are transferred from the SERF live-fish hauler to the net wells of FIM boats and transported to designated release sites. Using hand-held buckets, FSE and FIM staffs release the fish along shallow-water shorelines that have appropriate habitat. Short-term (24-hr) studies of the survival of red drum in all phases and tag retention of Phase II and Phase III fish are conducted in conjunction with each release event. At each release site, random subsamples of fish (100 Phase I, 50 Phase II, or 30 Phase III, depending on the stage of fish released) are placed separately by phase into cages at the time of release. The cages are checked for fish mortality 6 and 24 hours after release. Percent survival and CWT retention rates are determined and the lengths (SL and total length [TL]) and weights

(g) of all fish are recorded. When the survival rate of the caged fish is low, subsamples are provided to the AHG staff to evaluate possible reasons for the high mortality rate.

The FIM staff samples red drum from the admixed population in two ways. They use a standardized "stratified-random sampling" protocol similar to that used in the FMRI statewide sampling program (McMichael, 2000) and a "directed sampling" protocol in order to maximize the number of red drum captured.

Stratified-Random Sampling

The Alafia River sampling domain includes the area from the mouth of the river to a distance 4 nautical miles (nm) upriver in waters < 3 m deep, extending 16 m riverward from each shoreline (Fig. 2a). This sampling area is divided into 1-nm-latitude X 1-nm-longitude grids. Each grid is further subdivided into 100 microgrids (0.1 nm X 0.1 nm). The microgrids that incorporate a portion of the shoreline constitute the actual sampling units.

The FIM staff routinely samples this river; 21-m and 61-m haul seines are used to effectively catch red drum ranging in sizes approximately 25-50 mm SL STET 50-300 mm SL, respectively. The 21-m seine is made of 3-mm stretch-mesh nylon, has a center-bag with dimension 1.8-m depth X 1.8-m width X 1.8-m height and is set in water depths up to 1.8 m. The 61-m net is made of 25-mm stretch-mesh nylon, has a center-bag with dimension 3-m depth X 3-m width X 3-m height and is set in water depths up to 2.5 m. These nets are stretched between polypropylene lines that are fitted with evenly spaced lead weights at the bottom and flotation buoys at the top. Both are deployed by boat in a standardized elliptical shape parallel to the shoreline. The wings of each net are then brought together along the shoreline by hand and the bag is retrieved.

To date, red drum releases into the Alafia River have occurred in March-April (Phase II), June-July (Phase III), and December (Phase I) 2000 and in June-July (Phase III) and December (Phase I) 2001. Beginning in January 2000, FIM staff sampled red drum to estimate the relative abundance of wild red drum in the Alafia River prior to the stock-enhancement effort. Since the initiation of the stock enhancement experiment, FIM has sampled within 1-2 weeks after each release and monthly thereafter.

Following standard FIM protocol (McMichael 2000) sampling sites within each grid are randomly selected from the available shoreline microgrids. The sampling gear and number of samples collected each month varies depending on the size of red drum in the river at the time of sampling. For all collections, the sites sampled are evenly distributed among grids and between the north and south shorelines. For example, if 16 samples are collected with the 61-m haul seine, four samples are collected from each grid, and within each grid, two of these samples are collected from north-shoreline microgrids and two are collected from south-shoreline microgrids. When a chosen microgrid cannot be sampled because of gear or habitat constraints (a rare event), an alternate microgrid is selected in a standardized random fashion.

All samples are processed in the field immediately after collection. Most samples are processed according to standard FIM protocols (McMichael, 2000). For each sample, all individuals are identified to the lowest practical taxon and counted. For each taxon, the lengths (mm SL) of 10-40 individuals (depending on the size and total number of individuals in the taxon) are measured. All red drum (up to 100) in the cohorts that could include Phase II and Phase III fish are checked for CWTs by using a NMT V-detector.

Depending on the number of red drum in the collection, a subsample or the entire sample of red drum is retained from each collection that contains red drum. Each individual is double-checked for the presence of a CWT. Between 10 and 20 red drum per grid per month are delivered to the AHG staff for health evaluation. Fin clips for genetic analysis are taken from all red drum without CWTs. From those fish possessing CWTs, the tags are extracted and later read using a dissecting microscope.

Detailed water quality and habitat information are also recorded at each sampling location. These data include sample equipment identification, location, weather, water quality, habitat, and gear-specific information.

The Little Manatee River sampling domain includes the area from the mouth of the river to a distance approximately 5 nm upriver in water < 1.8 m deep, extending riverward approximately 5 m from each shoreline. The river is divided into lower, middle, and upper sections (Fig. 2b) each subdivided into a 1-nm latitude X 1-nm longitude grid. Both FIM and MML staffs sample this river.

Field sampling protocols are similar to those followed in the Alafia River except for the timing of post-enhancement sampling and the number of collections made each month. Fifty-eight locations per month are sampled for four months after a release. Thereafter, sampling effort is reduced by one-half

Figure 2b. Red drum (*Sciaenops ocellatus*) release and sampling domains. Insets show general location of each river in Florida. Release sites were designated as shorelines within each river section where suitable habitat was present. Little Manatee River; thick lines delineate the three river sections (lower, middle, upper) used in the stock enhancement experiment.

(29 locations) until the next hatchery release occurs, after which the sampling effort returns to 58 locations per month for the following 4 months.

Thus far, Phase I red drum have been stocked into the Little Manatee River during August 2000 and July, August, and September 2001. Sample-processing protocols also follow those described for the Alafia River monitoring, except for the following. Regardless of the level of monthly sampling effort, only two collections per river section are fully processed (all taxa identified and a subset of each taxon measured). In all other samples, only red drum and economically valuable species are processed. The laboratory procedures are as described for the Alafia River samples except that no red drum are provided to the AHG staff. Water quality and habitat data are also recorded as in the Alafia River sampling.
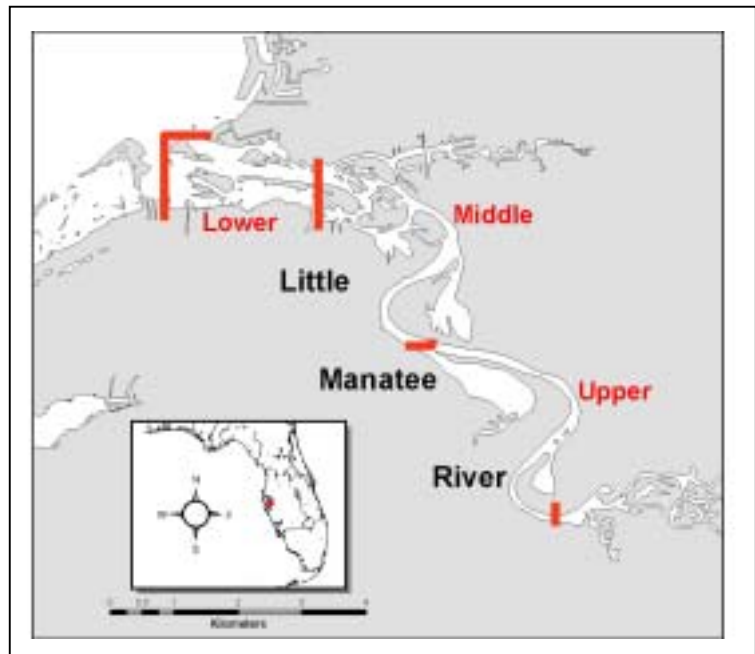
Directed Sampling

As juvenile red drum attain sizes > 200 mm SL, they migrate into deeper portions of the river or immigrate into other habitats in Tampa Bay (Peters and McMichael, 1987). Little is known of the movements and abundance of these large juvenile and subadult (200-400 mm SL) red drum. Historically it has been difficult to sample red drum in this size-class using standard FIM sampling techniques such as haul seines. To address this problem, FIM staff developed a directed red drum sampling program, which employs specialized gear for the capture of red drum in this size range. This sampling, in combination with the standard sampling described above, allows FIM staff to monitor red drum from the time that they are YOY until they enter the fishery (~400 mm SL).

Directed sampling is conducted monthly in both the Alafia and Little Manatee rivers and adjacent Tampa Bay waters. Sample area and habitat are not restricted. The staffs of FIM and MML search for red drum in a wide variety of habitats and locations using rod-and-reel gear, trammel nets, and ultrasonic telemetry. Sampling sites are selected by locating suitable habitat, by visually sighting red drum, or by using acoustic tracking devices to locate fish tagged with ultrasonic transmitters. The type of sampling gear used depends on the weather, tidal conditions, and accessibility of the habitat to be sampled. The sampling effort depends on the time of year, gear used, and availability of personnel. Directed sampling trips in which rods-and-reels or trammel-nets are used are often scheduled when tide levels allow access to shoreline habitats. Acoustic-tracking trips are conducted weekly following the release of red drum implanted with ultrasonic transmitters (U-tags; Sonitronics, Tucson, AZ). Tracking efforts continue based upon the life of the implanted U-tags and the number of red drum estimated to be in the vicinity. Light-tackle rod-and-reel gear, baited with live bait or artificial lures, is used in a variety of habitats. Trammel nets are used on the mud and grass flats and along some shoreline habitats. Monitoring of U-tagged red drum is conducted in release areas and in adjacent Tampa Bay waters that have habitats suitable for red drum. Additional information on U-tagged fish is provided by anglers who report fish captures.

The trammel nets are approximately 366 m long and 2.4 m deep, are constructed of monofilament netting (70-mm stretch-mesh inner wall and 305-mm stretch-mesh outer wall), and have a leaded bottom line and a floated top line. They are deployed from shallow-draft mullet skiffs such that they encircle and capture the fish. The net is quickly retrieved by hand to reduce fish mortality.

For acoustic tracking, the MML and FSE staffs surgically implant 200- to 550-mm SL red drum with the U-tags. These fish are stocked into the Alafia River at locations that have suitable shoreline habitats; there, they join schools of red drum in the vicinity. The U-tags emit a unique set of "pings" at pre-set frequencies, have a lifespan of approximately 18 months, and have a maximum signal range of approximately 500 m in open water. The means and variances for the six-month survival rate, surgical incision-healing rate, and U-tag retention rate for U-tagged red drum are known. The U-tagged red drum are monitored by using a hydrophone and receiver to listen for signals that help locate the fish. When U-tagged red drum are located, the following is recorded: transmitter code, latitude and longitude, bottom and shoreline habitat data, bottom and surface salinity, water temperature, dissolved oxygen level, and approximate number and size range of the observed fish. After the fish are located, MML and FIM staffs attempt to collect the fish using rods-and-reels and cast nets.

All collected red drum are counted, measured (SL), and checked for CWTs. Fish containing CWTs are taken to the laboratory where the CWTs are extracted and read. Fin clips from the second dorsal fin are removed from all red drum that do not have CWTs and are delivered to the BGL staff for genetic identification.

FDM and MML Angler Surveys

The FDM staff routinely and systematically interviews anglers to obtain catch (all fish caught) and harvest (fish retained for consumption) information at established locations such as public boat ramps, marinas, bridges, piers, jetties, beaches, and shores throughout Florida. They collect data on the number, size, and species of fish captured; time spent fishing; location of fishing effort; and species targeted. The MML staff supports the FDM efforts through a public-awareness advertising campaign. Because most hatchery-reared red drum are released into the Alafia and Little Manatee rivers without external tags, it is important to inform and enlist anglers to provide fin clips and fish-capture information for all red drum captured in Tampa Bay.

Sampling Design

For the red drum stock enhancement project, the FDM had available approximately 20 years of National Marine Fisheries Service Marine Recreational Fisheries Statistics Survey (MRFSS) data as background information on red drum harvested in Tampa Bay. During the 1.5- to 2.5-year lag between the first release of red drum in spring 2000 and the recruitment of hatchery-reared red drum into the fishery, FDM staff also did the following background work: (1) conducted a baseline assessment of the catch per unit effort (CPUE) of red drum, (2) gathered and analyzed basic statistics (mm SL and weight) on the captured fish prior to the appearance of the hatchery-reared red drum in the collective recreational angler catch (creel), and (3) fine-tuned the sampling protocol.

Initially, FDM examined the MRFSS angler-interview (angler-intercept) data for the Tampa Bay area from 1994 through 1998 to determine sites in Tampa Bay where red drum have been landed. A subset of these sites was selected based on proximity to release areas and the potential for intercepting anglers with red drum catches. The ontogenetically related movements of red drum were also considered in the site selection. As the hatchery-reared red drum increase in size, most of them gradually disperse downstream from their riverine release sites and move toward the open bay. Thus, most angler-intercept locations were selected from the eastern side of Tampa Bay, south of the Alafia and Little Manatee rivers (Fig. 1). However, red drum tagged and released on the eastern side of Tampa Bay have been subsequently captured on the western side of the bay (FMRI, unpublished data). Therefore, selected sites on the western side of the bay were also targeted for monitoring red drum harvested by anglers.

Upon examination of the 1994-1998 MRFSS survey, the FDM staff determined that the protocol for collecting those data was inadequate as the sole methodology for monitoring changes in red drum angler harvest resulting from the stock enhancement effort because insufficient numbers of red drum were being collected. The MRFSS is principally designed to provide catch estimates of all species caught by anglers on a state or regional level (Essig and Holliday, 1991). Therefore, to enable detection of statistical differences in red drum catch rates in the creel, FDM developed a sampling strategy

dedicated to providing a sufficient number of intercepts of those anglers who targeted or captured red drum.

Several problems were considered when designing this directed sampling strategy. Red drum harvest is limited to one fish 46-69 cm (18-27 in) TL per person per day. There are few sampling locations within Tampa Bay where anglers report high red drum catches. Details of the dispersal patterns of juvenile red drum from the Alafia and Little Manatee rivers into Tampa Bay are not well understood; thus, FDM staff would need to make assumptions regarding the proportion of hatchery-reared fish that would eventually be in the Tampa Bay red drum population. Moreover, the FDM 2000-2001 data on anglers that fish in Tampa Bay or use facilities in Tampa Bay to launch and retrieve their boats indicate that approximately 12.5% of these anglers target red drum, 5.0% catch and release red drum, and less than 3.0% harvest the species**.** Therefore, the estimated number of anglers that would need to be interviewed was daunting.

To improve the estimate of the number of angler intercepts and red drum needed to detect a contribution of hatchery-reared red drum to the creel, the FDM staff consulted MRFSS landings information to establish the proportion of anglers who harvested red drum over the five-year period 1994-1998. They used standard power calculations (Moher *et al.*, 1994; Zar, 1996) to estimate the minimum number of angler intercepts required to detect 10%, 25%, 50%, and 100% differences in red drum catch rates at the 95% confidence level with power levels of 80% and 90%. For example, FDM could be 90% certain of detecting a 50% difference in the catch rate of red drum 95% of the time if hatchery-reared red drum constituted 10% of the Tampa Bay red drum population. The FMD staff routinely uses the power calculations to determine the number and location of intercept sites to target. For this determination, the angler-intercept sites are examined individually and in combination.

To assign sampling days to angler-intercept sites in the directed red drum sampling project, FDM uses a weighted, random-sampling design. The probability of selecting a given site depends on angler activity; busier sites have higher probabilities of being selected than do sites where only a few anglers might be intercepted. To maximize the number of interviews with anglers who catch or harvest red drum, sampling locations are selected weekly to allow timely integration of newly identified sites into the sample pool and the removal of unproductive sites.

FDM staff assumes that the sample pool is dynamic and that fishing activity will change temporally and geographically within the bay. Changes in the number of locations targeted for interviews are done in a manner that minimally disrupts the sampling protocol and optimizes the possibility of gathering data.

An ancillary goal of this sampling activity is to examine the feasibility of this adaptive sampling design, which takes advantage of accrued information to adjust sampling decisions "on the fly" (Oehmke *et al.*, in press; Hardwick and Stout, 1998). Adaptive designs have the potential to produce exact solutions for sample allocation problems inherent in a species-directed fisheries-monitoring project such as the FDM portion of the Tampa Bay red drum project.

Sampling Protocol

For the MRFSS, FDM staff visits approximately 60% of the angler-intercept sites on weekends (Saturday and Sunday) and 40% on weekdays (all other days), reflecting the

increase in fishing pressure during the weekends. In contrast, for the directed red drum sampling, FDM staff visits 50% of the angler-intercept sites on the weekends and 50% on weekdays. Because each FDM staff member can effectively complete only four sampling assignments in a given week and because sites may be assigned only once on any given day, a 50%:50% division of weekend versus weekday angler-intercept site visits represents the most effective use of FDM staff time. If personnel are available, the number of sites visited per week is increased by up to 25%. These additional locations constitute a buffer and are not required to meet the minimum sampling requirements established by the power calculations.

For the directed red drum sampling at each angler-intercept site, an FDM staff member first screens anglers who have completed their fishing trips to determine if they targeted, caught and released, or harvested red drum. Anglers whose trips fall into one or more of these three categories are further questioned about the locations at which they fished, gear types they used, other species they harvested, the duration of their fishing trips, and fishing-group sizes. With their permission, the FDM staff member examines their finfish catches and weighs (kg) and measures (TL) their harvested fish. The harvested red drum are also scanned for the presence of CWTs using the NMT V-detector. If the fish possesses a CWT, the red drum carcass is requested for CWT extraction by FIM staff. From each red drum without a CWT, a fin clip is collected for genetic identification by BGL staff.

To improve the probability that adequate numbers of angler interviews are conducted and to expedite the detection of hatchery-reared red drum in the field, three FDM staff members are devoted to obtaining information on red drum catch in Tampa Bay. In addition, all FDM staff members who survey anglers in the Tampa Bay area in the MRFSS project collect similar data on red drum and angler effort and obtain red drum fin clips for genetic identification when possible.

Project Awareness

Recreational anglers are the principal recipients of the benefits of this project and their participation and involvement are important components of its success. To promote project awareness and engage angler participation, the MML and FSE staffs initiated an outreach campaign. The outreach program includes displaying posters and distributing information and fin-clip kits to bait-and-tackle shops, staging interviews with the media, and directly contacting anglers.

Together, the MML and FSE staffs designed a letter-sized poster advertising the project and soliciting angler assistance. The poster contains a color picture of a red drum, schematic drawings depicting how to take a fin clip from the second dorsal fin, the FWC "Redfish Hotline" phone number, and further instructions on participation. Laminated versions are posted in bait-and-tackle shops, boating supply centers, convenience stores, and marinas and at fishing piers, parks, and boat ramps. Non-laminated and postcard-sized versions are available for anglers to pick up at some of these locations and are provided to angler organizations. The postcard is also included in Fin Clip Kits, which are distributed to bait-and-tackle shops and angler organizations and contain the supplies and instructions needed for anglers to take tissue samples from red drum. Numerous bait-and-tackle shops serve as sites for anglers to obtain Fin Clip Kits and to deliver red drum tissue samples and/or carcasses.

AHG Fish Health Monitoring

A specific area of technical concern in stock enhancement research and implementation is fish health management (Pruder *et al.*, 1999). Fish encounter more stress in culture conditions than in the wild. Ideally, fish that are reared in aquaculture facilities are exposed to a minimum of pathogens, but they are susceptible to a number of infectious pathogens and parasites that thrive in closed systems (Landsberg, 1989; Landsberg *et al.*, 1994). Releasing diseased or pathogen-carrier fish into natural waters can have serious consequences for indigenous fish stocks (Goede, 1986). Additionally, if hatchery fish are in sub-optimal health before release, they are less likely to survive in the wild after release (Florida Department of Environmental Protection, 1995). Upon release, they may be compromised by parasites commonly found in and on wild fish. When artificial foods constitute a principal component of the diet of hatchery-reared fish, slow adaptation, or a failure to adapt, to the natural prey available in the various release habitats may affect the health or survival of those fish after their release. Any of these factors could influence the survival of the red drum prior to and after release and thereby affect the validity of the experiment. Therefore, the health history of each broodstock individual and hatchery-reared brood must be routinely monitored and documented throughout the breeding and rearing process to ensure with the highest probability possible that the fish are pathogen-free when they are released. Where appropriate, fish are treated prior to release to remove pathogenic parasites and to minimize the risk of parasite transfer to wild stocks (Landsberg *et al.*, 1991). Equally importantly, the admixed population must be routinely monitored before, during, and after release to evaluate any effects that the stock enhancement endeavor may have on the stocked fish or the recipient wild population.

The AHG staff developed an extensive health-screening protocol for evaluating fish. They grossly examine subsamples of each brood for signs of physical abnormalities, mechanical damage or disease. Using compound microscopes, they examine the body surface, gills and internal organs for parasites. They also culture a sample of the posterior kidney for bacteria. The AGH staff applied this protocol to wild red drum from Tampa Bay prior to the initiation of the stock enhancement experiment. They currently apply it to both hatchery-reared and wild red drum in each phase of growth collected from the admixed population by FIM during their post-enhancement sampling. The AHG staff objectives are to presume all fish healthy and pathogen-free at the time of release, to document changes in the health of stocked red drum as they adapt to the wild, and to compare the health and pathogen levels between stocked and wild red drum in the same cohorts and between the admixed and pre-release red drum populations.

Laboratory Protocol

The AHG staff monitors the ectoparasites *Ambiphyra* sp., *Amyloodinium ocellatum*, *Trichodina* sp., *Trichodinella epizootica*, and *Ergasilus* sp. and the endoparasites *Scolex polymorphus* and *Ceratomyxa* sp. These parasites are common to both wild red drum from Tampa Bay and hatchery-reared red drum from SERF. To determine the presence of gill parasites, the first left gill arch of each fish is excised and examined microscopically. The filaments are then cut from the gill arch and closely examined. Skin scrapes, obtained by scraping a glass coverslip along the length of the body, including the fins, are also examined microscopically for the presence of any external parasites. To examine the internal organs for the presence of parasites, the viscera are removed intact and fresh squash preparations from the liver, kidney, spleen,

anterior and posterior intestine, cecae, and gall bladder are viewed with compound microscopes (Landsberg *et al.*, 1998).

The AHG has identified and routinely monitors the bacterial flora of SERF hatchery-reared red drum both before and after their release and of wild red drum from Tampa Bay, including the Alafia River. To determine the presence and types of bacteria in the fish, a microbiologist using sterile techniques samples the posterior kidney. The presence of bacteria in the kidney is indicative of a systemic bacterial infection.

All tissues and organs evaluated for parasites and bacterial infections are also examined for other types of obvious physical abnormalities, mechanical damage, or disease. The fullness of the gall bladder and color of the bile are noted, as is the relative amount of mesenteric fat. The condition-factor (fish weight [gm] / fish length [mm SL^3] X 10^5), hepato-somatic index (liver weight / body weight X 100), and, if appropriate, gonado-somatic index (gonad weight / body weight X 100) are determined because they are indicators of the overall health and robustness of the fish. A portion of the liver is fixed in paraformaldehyde, embedded in plastic, sectioned (3-µm thickness), and thionin-stained in the FMRI histology laboratory for evaluation. Comparative percentage concentration of liver lipid is determined by gravimetric assay. The severed head, labeled according to collection-site designation, is provided to FIM staff to check for the presence of a CWT and extract the tag if it is present. Tissue samples or fin clips with appropriate collection data are provided to the BGL for genetic-tag analysis.

Sampling Protocol

Baseline health information on red drum has been collected during three projects: (1) a long-term AHG fish-health program for all species cultured at SERF, (2) a comprehensive study of the health of the Tampa Bay red drum population conducted in 1992-1993, which resulted in the development of the above protocol, and (3) an intensive sampling effort in the Alafia River for wild red drum, conducted by FIM staff during the two-week period prior to the first release of hatchery-reared red drum in early 2000. In the present stock-enhancement experiment, the FSE staff closely monitors red drum rearing to ensure good health. If problems occur, the AHG staff is immediately notified and investigates potential sources of the problem. One week to ten days prior to harvest, red drum samples are collected for independent health certification by the University of Florida's Institute of Food and Agricultural Sciences Tropical Aquaculture Lab (Ruskin, FL). At that time, a random sample of ten fish from each brood is also collected and evaluated by the AHG staff. The AHG staff evaluates another random sample of ten fish from each brood on the day of harvest and release. If the harvested fish are held longer in tanks for tagging, grading, acclimation, or health reasons, yet another random sample of ten fish is evaluated on the day of that release. The data obtained on that day are compared to previous data to check for changes in the overall health status of the fish during the stressful period of harvesting, tagging, and transportation. Fish that do not meet minimal health criteria are not released. These criteria include evidence of internal or external bacterial infection, detection of levels of parasites higher than normally found in or on wild red drum, low condition-factor, and the presence of external lesions or abrasions. The FSE attempts to correct the health problem and the AHG monitors the health of these fish until the problem is corrected and the fish are released.

Twenty-four hours after the fish are released, the FIM staff examines the fish from the net pens to monitor short-term tag retention and survival of the stocked fish. If mortality exceeds 5%, the AHG staff fully evaluates a sample of both moribund and apparently healthy fish using the laboratory protocol described above.

Shortly after each release of hatchery-reared fish, FIM collects a post-enhancement sample of hatchery-reared and wild red drum from the admixed population in the vicinity of the release area. These fish are evaluated for selected health criteria and to determine if the stocked individuals are more susceptible to wild pathogens and parasite infestations than are the wild fish. The captured fish are maintained alive and the sample from each collection is held in a separate container until they are processed. A maximum of ten red drum per grid, per sampling event (i.e., 40 fish per day) is collected for evaluation. The FIM or BGL staffs inform the AHG staff of the origin of each fish (wild or hatchery-reared) after they test for the presence of a CWT or genetically identify the individual.

The AHG staff uses the baseline red drum health data and the data obtained during this experiment to document the changes in the stocked red drum as they adapt to the local environment and the health effects of the stock enhancement experiment on the admixed red drum population. In the future, red drum that have entered the Tampa Bay fishery and are returned via FIM collections or FDM angler intercepts will be evaluated as is appropriate to assess the long-term health implications of stocking red drum into Tampa Bay.

Effort Involved in a Stock Enhancement Experiment

Perhaps the most unexpected surprise in conducting this stock enhancement experiment has been the tremendous amount of effort and coordination among relatively independent research groups that is necessary. In the 2.5 years since the initiation of this experiment, each group has put forth the effort and obtained the information described below.

In addition to the approximately 600 YOY wild red drum analyzed for both the mtDNA and microsatellite components of the genetic tag, the BGL staff has analyzed the mtDNA component of approximately 2,000 red drum from FIM staff post-enhancement collections and 250 red drum from MML staff post-enhancement collections. Of these individuals, the BGL staff has analyzed, for the battery of microsatellite loci, 230 red drum with mtDNA genetic-tag genotypes that matched those of broodstock mothers. Because most hatchery-reared red drum are released in Phase I, the total number of red drum analyzed for this experiment is expected to double. To reduce the effort and time involved in genetic tag analysis, the BGL staff recently eliminated the mtDNA component and organized the microsatellite analysis in such a way that a preliminary analysis of four loci in one multiplex reaction can be used to screen for the origins of individuals with about 90% accuracy. Those with genotypes consistent with hatchery origin are further evaluated for the remaining five loci, again in multiplex reactions. This strategy eliminates the need to separate broodstock females according to the rarity of their mtDNA haplotypes. Because one goal of this experiment is to measurably increase the average catch of red drum in an angler's creel beyond the pre-enhancement level (i.e., the level that existed when the Tampa Bay fishery was based solely on wild fish), this genetic analysis will continue for a number of years after the stocking component of the experiment is completed.

In seven separate release events during 2000-2001, FSE released approximately 1,242,000 hatchery-reared red drum into the Alafia (334,000 fish) and Little Manatee (910,000 fish) rivers. These fish were spawned from 15 different broodstock groups composed of a total of 28 females and 34 males. Approximately 344,000 and 800,000 fish were released in 2000 and 2001, respectively. Of these, over 1,150,000 were released as Phase-I red drum (242,000 into the Alafia River and 900,000 into the Little Manatee River). Approximately 45,000 of the 62,400 red drum reared to Phase II were released in into the Alafia River in 2000 and nearly 22,700 of the 30,000 red drum reared to Phase III were released into the Alafia River in 2001. The research plan states that 1,360,000 red drum per year will be released, in the ratio of 88% in Phase I, 9% in Phase II, and 3% in Phase III. These are projected numbers of fish. Of course, practical challenges of various types influence the actual number and phase of the fish released.

In fulfilling its obligation to collect an adequate number of red drum from Tampa Bay in pre- and post-release samples, the FIM group has made a total of more than 850 seine hauls in the two rivers targeted as release sites. Of those, approximately 80% were conducted in the Alafia River, one-third with the 21-m haul seine and two-thirds with the 61-m haul seine. The FIM staff has captured a total of approximately 2,000 red drum, almost 150 of which were tagged with CWTs. The FIM directed-sampling program was initiated in 2001, when FIM staff anticipated that stocked hatchery-reared red drum had grown large enough to easily avoid haul seines. In the Alafia River and adjacent Tampa Bay waters during 2001, FIM staff sampled nearly 40 sites using trammel nets and more than 125 sites using rod-and-reel gear. Also during 2001, the FSE and MML staffs U-tagged and released more than 90 sub-adult red drum in three groups of fish. The FIM and MML staffs made nearly 150 field trips to search for these fish. Many of the U-tagged red drum were collected by these scientists and several more were caught by recreational fishermen in several areas of Tampa Bay.

Since October 2001, the MML staff has conducted nearly 200 21-m haul seines as part of their assessment in the Little Manatee River. These samples contained a total of 320 red drum. Similar to the Alafia River collections made by FIM staff, most of the red drum were collected from upriver portions of the study area. From their fisheries-independent sampling effort in the Little Manatee River, MML staff has provided the BGL staff with more than 325 fin clips from captured red drum. Since the initiation of the targeted effort to obtain information on the red drum fishery and find U-tagged fish in anglers' catches, MML staff has made more than 27,000 angler intercepts in the Tampa Bay region and has obtained catch data from 722 anglers. Over 500 red drum were tested for U-tags.

The AHG staff has evaluated nearly 1,000 red drum (more than 300 hatchery-reared fish, 200 stocked fish, and 300 wild fish) for target-parasite prevalence, condition-factor, and hepato-somatic indices. Approximately 9,000 fresh squash preparations of fish tissue have been examined microscopically for parasites and tissue abnormalities. Posterior kidneys from more than 900 fish have been cultured to search for systemic bacteria infections. More than 900 samples of liver from red drum were histologically prepared for microscopic evaluation. Of these, approximately 500 will be evaluated for lipid content.

Of course, a stock-enhancement experiment of this magnitude requires substantial personnel involvement of both full- and part-time employees and, in this case, also recreational anglers. To conduct the genetic analysis and maintain the genetics database, three full-time and three half-time staff members work on various components of the project. To manage the broodstock and rear the broods, 15 full-time FSE staff members are involved. To conduct the pre- and post-enhancement fisheries-independent field collecting and U-tag tracking, the FIM

program uses four full-time staff members. To query anglers and obtain fishery-related information on the red drum harvest in the Tampa Bay region, the FDM program uses the equivalent of four full-time staff members. However, only three of these individuals are dedicated to obtaining information on the red drum fishery. All others obtain information on red drum as a component of general angler-intercept surveys. To monitor the health of the red drum at SERF and evaluate the health status of fish captured in the pre- and post-release samples provided by the FIM staff, four full-time and one half-time AHG staff members work in the laboratory and one full-time staff member works at SERF. In addition to the various hatchery, field, and laboratory personnel, a total of twelve supervisors work part-time to oversee and manage the project.

Stock Enhancement Monitoring-Worth the Effort ?

Wild-population fisheries are seriously declining worldwide (Botsford, *et al.*, 1997; Vitousek, *et al.*, 1997; Pauly, *et al.*, 1998). Three methods are commonly used to attempt the replenishment of depleted stocks: regulating fishing effort, restoring habitats critical to one or more life stages of the stock, and artificially supplementing the reproductive population through restoration or enhancement programs (Leber and Lee, 1997). Stock restoration or enhancement is gaining increased popularity and is practiced at various levels worldwide, but it is generally not closely monitored or evaluated (Welcomme and Bartley, 1998). A major problem in justifying the expense and effort associated with stock enhancement is determining if it is successful. Leber (1999) points out that success has typically been measured by production levels and numbers of fish stocked. However, the success of a stock enhancement endeavor should be evaluated according to the goals of the project. The goals are often defined as the measurable contribution to the fishery or to the reproductive population. Leber (1999) states that in addition to estimating the increase in the size of the enhanced population or the increase in its reproductive output, the focus also should be on determining if the stocked fish are simply replacing the wild fish.

The emphasis on production as the principal measure of success has been maintained because after hatchery-reared fish are released, it is difficult – or, when the stocked fish are released as eggs, larvae, or small fry, it is impossible – to track the stocked fish or to distinguish them from wild fish. Various methods of estimating the success of stock-enhancement efforts have been used. For example, to estimate the contribution of stocked brown trout (*Salmo trutta*), researchers in Denmark used shifts in the frequencies of 'local' native mtDNA genotypes over time and comparisons of the frequencies of local versus non-local mtDNA genotypes in rivers undergoing stocking with those frequencies in rivers that had been stocked at different times in the past (Ruzzante *et al.*, 2001). Researchers in Hawaii used increases in the percentage of Pacific threadfin (*Polydactylus sexfilis*) in fishermen's creels (Friedlander and Ziemann, in press). Japanese researchers used increases in catch statistics versus number of 'seeds' released to measure bay scallop (*Argopecten irradians*) stocking success (Kitada and Fujishima, 1997). However, none of these methods unambiguously define the level of contribution that stock-enhancement efforts have made, nor do they demonstrate that the methodology used in releasing the fish provides the hatchery-reared fish the best opportunity for survival.

Release conditions are clearly important in determining the survival of stocked fish. To maximize the probability that stocked fish survive until they contribute to the ultimate objective of the stock-enhancement project, the best release conditions must be known and followed (Leber, 1999). Size-at-release, location of release, and timing of release can each influence

survival of the stocked fish, and these factors can all work synergistically to influence survival (Stoner and Davis, 1994; Leber *et al.*, 1996; Leber *et al*., 1998; Leber, 1999). The best stocking conditions can be determined only through an experimental approach in the initial stages of the stock enhancement endeavor. In addition, other factors--such as the similarity or dissimilarity between the genetic composition of the stocked fish and the recipient wild-fish population, the health of the stocked fish at the time of release, the degree of handling-induced stress, and the carrying capacity of the environment for the targeted fish species--all have influenced the success of stock enhancement programs (Vea Salvanes *et al.,* 1995; Bell and Gervis, 1999; Kuwada, *et al.*, 2000; Ashford and Danzmann, 2001; Fushimi, 2001; Rasmussen and Geertz-Hansen, 2001).

In the Tampa Bay red drum stock-enhancement program, we have attempted to consider all of these factors in rearing and releasing red drum, and through our genetic-tag- and CWT-based monitoring programs, we should be able to estimate the contribution of stocked red drum to the fishermen's creel. Finally, our detailed accounting of expenditures throughout the rearing process and documentation of the contribution of the stocked fish to the creels of fishermen should enable us to estimate the per-fish cost of this stock-enhancement project. Few marine stocking programs have been monitored for their economic success (Hilborn, 1998).

Studies such as the one described here are huge in scale, are complex, and require the coordination of many diverse research groups. Ideally, all stock enhancement projects would incorporate the research components described here. Obviously, that is impossible for smaller-scale stock-enhancement endeavors. Nevertheless, experimentation with release conditions and attention to the culture conditions, genetic composition, and health of the stocked fish should always benefit a stock-enhancement effort. Because stock enhancement will probably continue to increase in popularity as a remedial method for supplementing depleted fish stocks, this approach could be subjected to increased scrutiny for both its ecological and genetic impacts on wild populations and its economic cost-to-benefit ratio. Thus, the experimental approach to stock enhancement will become increasingly important.

Acknowledgments

Literature Cited

Arnold, C. R., W. H. Bailey, T. D. Williams, A. Johnson, and J. L. Lasswell. 1977. Laboratory spawning and larval rearing of red drum and southern flounder. *Proc. Annu. Conf. Southeastern Assoc. Fish Wildl. Agencies* 31:437-440.

Ashford, B. D. and R. G. Danzmann. 2001. An examination of the post-planting success of hatchery brook char possessing different mitochondrial DNA haplotypes. *J. Fish Biol*. 59:544-554.

Bell, J. D. and M. Gervis. 1999. New species for coastal aquaculture in the tropical Pacific: constraints, prospects, and considerations. *Aquacult. Int*. 7:207-223.

Bert, T. M., M. D. Tringali, and J. Baker. 2001. Considerations for sustainable aquaculture, biodiversity, and ecosystem processes-a genetics perspective. In: Proceedings of the international conference on agriculture science and technology: promoting global innovation of agricultural science and technology and sustainable agriculture development. Vol. 3: Resources and environment. November 6-9, Beijing, China (Organizing Committee, ICAST, eds.), p. 238-254. ICAST, Beijing, China. (Copies are available from the authors.)

Bert, T. M., M. D. Tringali, and S. Seyoum. 2002. Development and application of genetic tags for ecological aquaculture. In: Ecological aquaculture (B. Costa-Pierce, ed.), p. 47-76. Blackwell Scientific Publishers, Ames, IA.

Botsford, L. W., J. C. Castilla, and C. H. Peerson. 1997. The management of fisheries and marine ecosystems. *Science* 277:500-515.

Brenner, C. 1983. Calculation of paternity index. In: Inclusion probabilities in parentage testing (R. Walker, ed.), p. 633-638. American Association of Blood Banks, Arlington, Virginia.

Colura, R. L., B. T. Hysmith, and R. E. Stevens. 1976. Fingerling production of striped bass (*Morone saxatilis*), spotted seatrout (*Cynoscion nebulosus*), and red drum (*Sciaenops ocellatus*) in saltwater ponds. *Proc. World Maricult. Soc.* 7:79-92.

Essig, R. J. and M. C. Holliday. 1991. Development of a recreational fishing survey: the marine recreational fisheries statistics survey case study. *Am. Fish. Soc. Symp*. 12:245-254.

Florida Department of Environmental Protection. 1995. Marine fish stock enhancement & hatchery legislative report. Florida Marine Research Institute, Florida Department of Environmental Protection. Rep. No. IHR1995-001. 77 p.

Friedlander, A. M. and D. A. Ziemann. Impact of hatchery releases on the recreational fishery for Pacific threadfin (*Polydactylus sexfilis*) in *Hawaii. Fish. Bull*., in press.

Fushimi, H. 2001. Production of juvenile marine finfish for stock enhancement in Japan. *Aquaculture* 200:33-53.

Graves, J. E. 1998. Molecular insights into the population structure of marine fishes. *J. Hered*. 89:427-437.

Geode, R. W. 1986. Management considerations in stocking of diseased fish or carrier fish. In: Fish culture in fisheries management (R. H. Stroud, ed.), p. 349-355. American Fisheries Society Special Publications, Bethesda, MD.

Hardwick, J. P. and Q. F. Stout. 1998. Flexible algorithms for creating and analyzing adaptive sampling procedures. *Inst. Math. Stat. Lec. Notes – Monogr. Ser.* 34:91-105.

Hilborn, R. 1998. The economic performance of marine stock enhancement projects. *Bull. Mar. Sci.* 62:661-674.

Hillis, D. M., B. K. Mable, A. Larson, S. K. Davis, and E. A. Zimmer. 1996. Nucleic acids IV: sequencing and cloning. In: Molecular systematics, 2nd ed. (D. M. Hillis, C. Moritz, and B. K. Mable, eds.), p. 321-381. Sinauer Associates, Inc., Sunderland, MA.

Kitada, S. and H. Fujishima. 1997. The stocking effectiveness of scallop in Hokkaido. *Nippon Suisan Gakkaishi* 63:686-693.

Kuwada, H., R. Masuda, S. Shiozawa, T. Kogane, K. Imaizumi, and K. Tsukamoto. 2000. Effect of fish size, handling stresses and training procedure on the swimming behavior of hatchery-reared striped jack: implications for stock enhancement. *Aquaculture* 185:245-256.

Landsberg, J. H. 1989. Parasites and associated diseases of fish in warm water-culture, with special emphasis on intensification. In: Fish culture in warm water systems: problems and trends. (M. Shilo and S. Sarig, eds.), p. 195-252. CRC Press, Boca Raton, FL.

Landsberg, J. H., B. A. Blakesley, R. O. Reese, G. McRae, and P. R. Forstchen. 1998. Parasites of fish as indicators of environmental stress. *Environ. Monit. Assess*. 51:211-232.

Landsberg, J. H., K. A. Steidinger, B. A. Blakesley, and R. L. Zondervan. 1994. Scanning electron microscope study of dinospores of *Amyloodinium* cf. *ocellatum*, a pathogenic dinoflagellate parasite of marine fish, and comments on its relationship to the Peridinales. *Dis. Aquat. Org*. 20:23-32.

Landsberg, J. H., G. K. Vermeer, S. A. Richards, and N. Perry. 1991. Control of the parasitic copepod *Caligus elongatus* on pond-reared red drum. *J. Aquat. Anim. Health* 3:206-209.

Leber, K. M. 1999. Rationale for an experimental approach to stock enhancement. In: Stock enhancement and sea ranching (B. R. Howell, E. Moksness, and T. Svåsand, eds.), p. 63-75. Blackwell Science Ltd., Oxford, UK.

Leber, K. M., S. M. Arce, D. A. Sterritt, and N. P. Brennan. 1996. Marine stock enhancement potential nursery habitats of striped mullet, *Mugil cephalus*, in Hawaii. *Fish. Bull.* 94:452-471.

Leber, K. M., N. P. Brennan, and S. M. Arce. 1998. Recruitment patterns of cultured juvenile Pacific threadfin, *Polydactylus sexfilis* (Polynemidae), released along sandy marine shores in Hawaii. *Bull. Mar. Sci.* 62:389-408.

Leber, K. M. and C.-S. Lee. 1997. Marine stock enhancement potential with striped mullet, *Mugil cephalus*, in Hawaii. *Bull. Natl. Res. Inst. Aquac. 0* (Suppl. 3):117-134.

McMichael, R. H., Jr. 2000. Fisheries independent monitoring program operating procedure manual. Rep. No. IHR2000-005, Florida Marine Research Institute, St. Petersburg, Florida. 256 p.

Merilä, J., M. Björklund, and A. J. Baker. 1997. Historical demography and present day population structure of the greenfinch, *Carduelis chloris* – an analysis of mtDNA control-region sequences. *Evolution* 51:946-956.

Moher, D., C. S. Duhlberg, and G. A. Wells. 1994. Statistical power, sample size, and their reporting in randomized controlled trials. *J. Am. Med. Assoc*. 272:122-124.

Murphy, M. D., and R. E. Crabtree. 2001. Changes in the age structure of nearshore adult red drum off west central Florida related to recruitment and fishing mortality. *N. Am. J. Fish. Manag.* 21:671-678.

Oehmke, R., J. Hardwick, and Q. F. Stout. Scalable algorithms for adaptive statistical designs. Scientific programming. IOS Press, Amsterdam, The Netherlands, in press.

Pauly, D., V. Christensen, J. Dalsgaard, R. Froese, and F. Torres, Jr. 1998. Fishing down marine food webs. *Science* 279:860-863.

Peters, K. M. and R. H. McMichael. 1987. Early life history of the red drum, *Sciaenops ocellatus* (Pisces: Sciaenidae), in Tampa Bay, Florida. *Estuaries* 10:92-107.

Pruder, G. D., D. A. Ziemann, K. L. Leber, J. L. Lotz, W. E. Hawkins, and L. D. Conquest. 1999. U.S. Gulf of Mexico marine stock enhancement five-year program: 1999-2004. Program for management for planning, execution, review, and evaluation. *Bull. Natl. Res. Inst. Aquacult., Suppl.* 1:89-99.

Rasmussen, G. and P. Geertz-Hansen. 2001. Fisheries management in inland and coastal waters in Denmark from 1987 to 1999. *Fish. Manag. Ecol.* 8:311-322.

Roberts, D. E., Jr., B. V. Harpster, and G. E. Henderson. 1978. Conditioning and induced spawning of the red drum (*Sciaenops ocellatus*) under varied conditions of photoperiod and temperature. *Proc. Annu. Meet. World Maricult. Soc.* 9:311-332.

Ruzzante, D. E., M. M. Hansen, and D. Meldrup. 2001. Distribution of individual inbreeding coefficients, relatedness and influence of stocking on native anadromous brown trout (*Salmo trutta*) population structure. *Mol. Ecol.* 10:2107-2128.

Saiki, R. K., D. H. Gelfand, S. Stoffel, S. Scarf, R. Higuchi, R. Horn, K. B. Mullis, and H. A. Erlich. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239:487-491.

Seyoum, S., M. D. Tringali, T. M. Bert, D. McElroy, and R. Stokes. 2000. An analysis of genetic population structure in red drum (*Sciaenops ocellatus*) based on mtDNA control region sequence. *Fish. Bull.* 98:127-138.

Stoner, A. W. and M. Davis. 1994. Experimental outplanting of juvenile queen conch, *Strombus gigas*: comparison of wild and hatchery-reared stocks. *U.S. Natl. Mar. Fish. Serv. Fish. Bull.* 92:390-411.

Turner, T. F., L. R. Richardson, and J. R. Gold. 1998. Polymorphic microsatellite DNA markers in red drum (*Sciaenops ocellatus*). *Mol. Ecol.* 7:1771-1788.

Vea Salvanes, A. G., D. Aksnes, J. Helge Fossa, and J. Giske. 1995. Simulated carrying capacities of fish in Norwegian fjords. *Fish. Oceanogr.* 4:17-43.

Vitousek, P. M., H. A. Mooney, J. Lubchenco, and J. M. Meilillo. 1997. Human domination of earth's ecosystems. *Science* 277:494-499.

Welcomme, R. L. and D. M. Bartley. 1998. Current approaches to the enhancement of fisheries. *Fish. Manag. Ecol.* 5:351-382.

Wilson, A. C., R. L. Cann, S. M. Carr, M. George, U. B. Gyllensten, K. M. Helmbychowski, R. G. Higuchi, S. R. Palumbi, E. M. Prager, R. D. Sage, and M. Stoneking. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. *Biol. J. Linn. Soc.* 26:375-400.

Zar, J. H. 1996. Biostatistical analysis. Simon and Schuster, Upper Saddle River, NJ.

■ III. PHYSIOLOGICAL AND ECOLOGICAL APPLICATIONS

Predation on Juvenile Chum Salmon *Oncorhynchus keta* by Fishes and Birds in Rivers and Coastal Oceanic Waters of Japan

Kazuya Nagasawa
Nikko Branch, National Research Institute of Aquaculture
Fisheries Research Agency, Chugushi, Nikko
Tochigi 321-1221
JAPAN
Email: ornatus@fra.affrc.go.jp

Hiroshi Kawamura
Kumaishi Branch
Hokkaido Fish Hatchery
Ayukawa 189-43, Kumaishi
Hokkaido 043-0402
JAPAN
Email: kawamurah@fishexp.pref.hokkaido.jp

Abstract

The present paper compiles information on predation by fishes and birds on juvenile chum salmon (*Oncorhynchus keta*) in rivers and coastal oceanic waters of Japan. In Japan, nearly 100% of chum salmon juveniles are reared at hatcheries and released into rivers. Various freshwater fishes, such as sculpins, gobiids and Japanese dace (*Tribolodon hakonensis*) are known to feed on released chum salmon juveniles. In this paper, detailed information is given about predation by fluvial sculpin (*Cottus nozawae*) in a river of southern Hokkaido. Evidence shows that sculpin preyed on chum salmon juveniles, but their impact on the population was low. Gulls and other birds aggregate at river-mouths during the season of chum salmon release and feed heavily on juveniles. A recent survey on the impact of avian predation on the chum salmon population in a river of western Hokkaido has shown that over 10% of released juveniles were consumed by gulls (black-tailed gulls *Larus crassirostris* and slaty-backed gulls *L. schistisagus*) for nine days after release, indicating that the gulls are significant predators. Gulls are the most abundant seabirds in coastal waters of Hokkaido, and their predation may be present in coastal oceanic waters as well. Night releases are highly recommended to reduce loss of chum salmon by avian predation in river-mouth regions.

Natural mortality is one of the most important factors controlling fish populations, and predation is a major source of natural mortality for juvenile Pacific salmon (Ruggerone, 1986; Wood, 1987; Rieman *et al.*, 1991; Ruggerone and Rogers, 1992; Beamish *et al.*, 1992; Collis *et*

al., 2001). In Japan, nearly 100% of juvenile chum salmon *Oncorhynchus keta* are reared at hatcheries and currently about two billion fish are released into rivers every spring (Kaeriyama, 2000). These juveniles travel to the sea and migrate along the coast of northern Japan to the southern Sea of Okhotsk (Fig. 1). Life history information on distribution, migration and feeding patterns is available, but little is known about the species' natural mortality, especially predation.

Nagasawa (1998) regarded the following nine fish species and two seabirds as major predators of juvenile chum salmon in coastal oceanic waters of Japan: Japanese dace *Tribolodon hakonensis*, Far Eastern dace *T. brandti*, white-spotted charr *Salvelinus leucomaenis*, Japanese halibut *Paralichthys olivacaeus*, Japanese sea perch *Lateolabrax japonicus*, spiny dogfish *Squalus acanthias*, arabesque

Figure 1. Migration route of juvenile chum salmon in coastal oceanic waters of northern Japan (modified from Irie, 1990), showing the locations of the Nishibetsu River (1), Memu River (2), Utabetsu River (3), Shokanbetsu River (4), Furuu River (5), Otsuchi River (6), Katsugi River (7), and Shou River (8).

greenling *Pleurogrammus azonus*, pink salmon *O. gorbuscha*, masu salmon *O. masou,* rhinoceros auklets *Cerorhinca monocerata* and black-tailed gulls *Larus crassirostris*. This paper reviews past literature and adds new research on fish and avian predation on chum salmon juveniles in Japanese rivers.
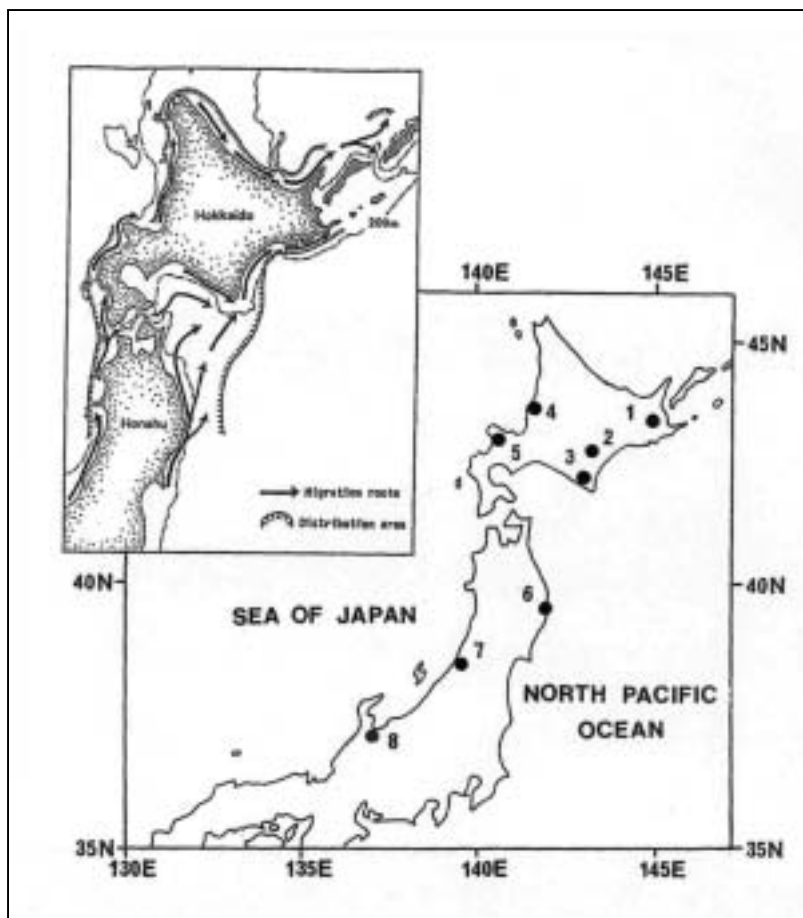
Predation in Rivers

There are several papers dealing with predation by white-spotted charr on chum salmon juveniles in Japanese rivers (Kubo, 1946; Hikita *et al.*, 1959; Takami and Nagasawa, 1996). In northern Japan, white-spotted charr are widely distributed and abundant in mountain streams and upper reaches of rivers. In eastern Hokkaido, Kunashiri and Iturup islands, Kubo (1946) found an average of 8.2 juveniles per stomach and regarded white-spotted charr as the most significant

predator. Many years later Takami and Nagasawa (1996) reported chum salmon in the stomachs of four (12.5%) out of 32 white-spotted charr in the Furuu River, western Hokkaido during the seaward migration period of both species (Fig. 1).

In Japan, masu salmon spend their first one to two years in rivers before migrating to the sea. During this time and during migration they prey heavily on juvenile chum salmon (Kubo, 1949; Hikita *et al.*, 1959). Tago (1994) reported the same in the Shou River of central Honshu (Fig. 1). Juvenile chum salmon are released from hatcheries during their natural migration period, late winter to early spring. In Tago's study the percentage of masu salmon ingesting chum salmon began to increase in mid- to late March, peaked in mid-April, and thereafter decreased (Fig. 2).
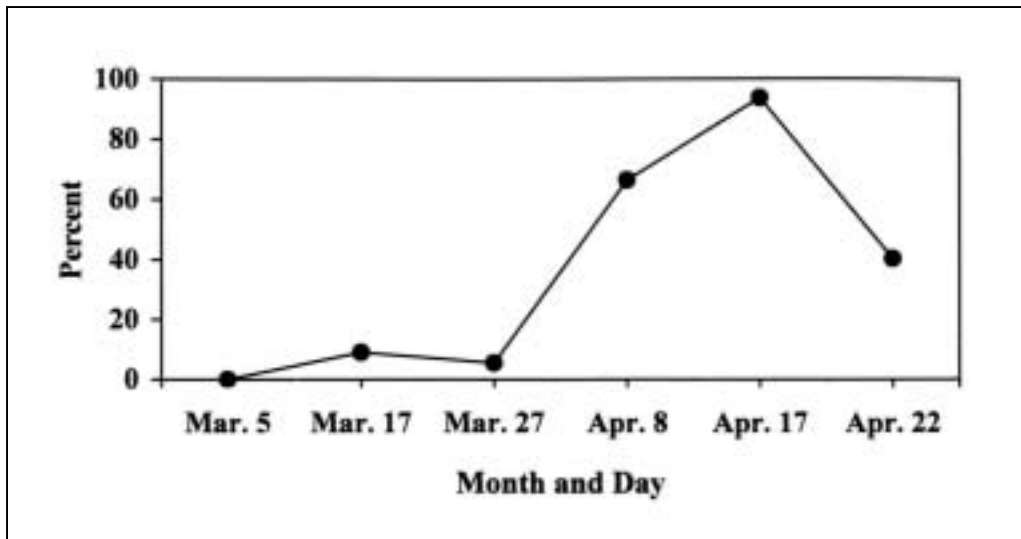

Figure 2. Seasonal changes in percent weight of chum salmon in the stomach contents of juvenile masu salmon from the Shou River, central Honshu, from early March to late April 1992 (original, raw data from Tago, 1994).

This trend corresponded with seasonal changes in abundance of sea-migrating chum salmon at sampling sites. In mid-April, a single chum salmon was usually preyed upon by one masu salmon.

Other salmonids known to feed on juvenile chum salmon in Japanese rivers include Dolly Varden *Salvelinus malma* (Kubo, 1946), Sakhalin huchen *Hucho perryi* (Kubo, 1946; Nakano, 1992), brook trout *Salvelinus fontinalis* and rainbow trout *Oncorhynchus mykiss* (Kubo, 1946; Hikita *et al.*, 1959). Of these species, Sakhalin huchen and Dolly Varden have such small populations in restricted rivers that the impact of their predation on chum salmon populations is minimal. Brook trout and rainbow trout populations are also low because they usually do not breed in northern Japan, suggesting a minimal impact. However, the latter two salmonids are known to reproduce abundantly and feed on chum salmon in some rivers of Hokkaido, such as the Nishibetsu River (Fig. 1 - Kubo, 1946; Hikita *et al.*, 1959). Japanese dace are also known predators, although insignificant in rivers (Inukai, 1949).

The most well studied predator of juvenile chum salmon in Japanese rivers is the fluvial sculpin *Cottus nozawae* (Hikita and Nagasawa, 1960; Kawamura, 1980, as *C. pollux;* Nagata, 1984; Nagata and Miyamoto, 1986). Fluvial sculpin mainly occur in the lower and middle reaches of rivers in Hokkaido and northern Honshu. They feed on a wide variety of organisms, such as aquatic insects, benthic animals, small fish and salmon eggs (Goto, 1989). Predation on chum salmon by fluvial sculpin has been studied in two rivers, the Memu River and Utabetsu River, Hokkaido (Fig. 1). Detailed information is given in the next section.

Another species of the freshwater sculpin, *C. hangiongensis*, has been reported to prey on chum salmon in the Otsuchi River, northern Honshu (Fig. 1 - Hiyama *et al.,* 1972a; 1972b). In April of 1964-65, juveniles were found in the stomachs of sculpin 13.2-35.7% of the time. At least 68 juveniles were found in the stomachs of 48 sculpin (1.4 salmon per sculpin).

The floating goby *Chaenogobius urotaenia* and Japanese trident goby *Tridentiger obscurus* are predators of chum salmon juveniles in Japan (Hiyama *et al.*, 1972a; 1972b; Amida and Okada, 1973). Information on predation by these gobies is very limited and there are only a few records from two rivers. In the Otsuchi River, northern Honshu, 29 (3.5%) out of 823 floating gobies and 2 (6.5%) out of 31 trident gobies collected in April had 35 (mean 1.2) and 3 (1.5) chum salmon in the stomachs, respectively (Hiyama *et al.*, 1972b). Amida and Okada (1973) reported that floating gobies actively fed on chum salmon at night in the Katsugi River, central Honshu (Fig. 1). In late April, over 40% of the gobies sampled were found to have ingested juveniles.

No scientific information is available on avian predation on juvenile chum salmon in Japanese rivers, except the river-mouth regions. However, Sakurai (1984) states in his book that two species of kingfishers, crested kingfisher *Ceryle lugubris* and common kingfishers *Alcedo atthis*, prey on sea-migrating chum salmon juveniles in eastern Hokkaido. These birds are frequently observed along streams in northern Japan. Sakurai also mentions that black-backed wagtails *Motacilla alba* and brown-headed thrushes *Turdus chrysalaus* feed on juveniles. Since brown dippers *Cinclus pallasii* are known to eat juvenile rainbow trout stocked into rivers (Hiyama *et al.*, 1960) and occur widely in Japan, including the northern region, where chum salmon propagation has been extensively conducted, predation by this bird species on chum salmon is very likely.

Case Studies on Predation by Fluvial Sculpin

Various aspects of fluvial sculpin biology (Kawamura, 1979, as *C. pollux*) and predation (Nagata, 1984) were investigated in the Utabetsu River, southern Hokkaido. Kawamura (1980) and Nagata and Miyamoto (1986) conducted intense studies in 1978-80 and 1983-84, respectively. The Utabetsu River is mere 13 km long and drains directly into the North Pacific Ocean. Chum salmon are released in spring (from mid-April to early June, usually in late May) from a governmental hatchery located about 4 km upstream from the river mouth. The number of the fish released in 1978-80 and 1983-84 ranged between 1.45 and 3.27 million. The juveniles do not remain in this river long. About 50% enter the sea by the next day and the remainder by the following 12-24 days.

The abundance of fluvial sculpin varied by sampling location. The species was abundant (0.15 fish/m^2) near the river mouth, but much less abundant (0.02-0.05 fish/m^2) in the upper stream. The sculpin were collected using cast and dip nets in May 1983 and comprised six size groups. Fish of size group 2 were the most abundant (40.6%) at 6.70 cm in mean body length (BL), followed by those of size groups 3 and 4 (26.7%, 8.95 cm BL and 11.7%, 10.75 cm BL,

respectively). The largest sculpin collected measured 17.0 cm BL. These percentages were used to estimate the density of fluvial sculpin occurring in May 1983. A total of 1200 fish were estimated to occur throughout the two river regions (river-mouth region and upper-stream region to the hatchery). In addition, the satiation weight of prey (Y_w) was determined by the body length (X) of predator, as shown by the linear equation:

$$Y_w = 0.695 \ X \ 0.695 \ X - 4.381 \qquad (1)$$

Feeding behavior of fluvial sculpin was also observed in a tank for seven days after the fish were satiated with chum salmon juveniles. The sculpin fed on few juveniles for the first three days, but fed actively for the remaining four days. The ratio of fish eaten in seven days to initial fish consumed for satiation decreased exponentially with an increase in sculpin body length. The relationship between ratio (Y) and body length (X) is shown by the formula:

$$Y = 111.902X^{-1.8201} \qquad (2)$$

The percentage of fluvial sculpin ingesting chum salmon juveniles varied between years (27.9-72.2%), with larger sculpin ingesting a greater number (Fig. 3). As many as eight chum salmon were recovered from two large fish (13 and 14 cm BL), and an average of about 2.4 juveniles were found in a single stomach. The chum salmon found in stomachs were smaller than the released fish (e.g., 0.87 and 0.92 g for the 1983 and 1984 mean BW of ingested juveniles vs 0.92 and 1.29 g for released juveniles).
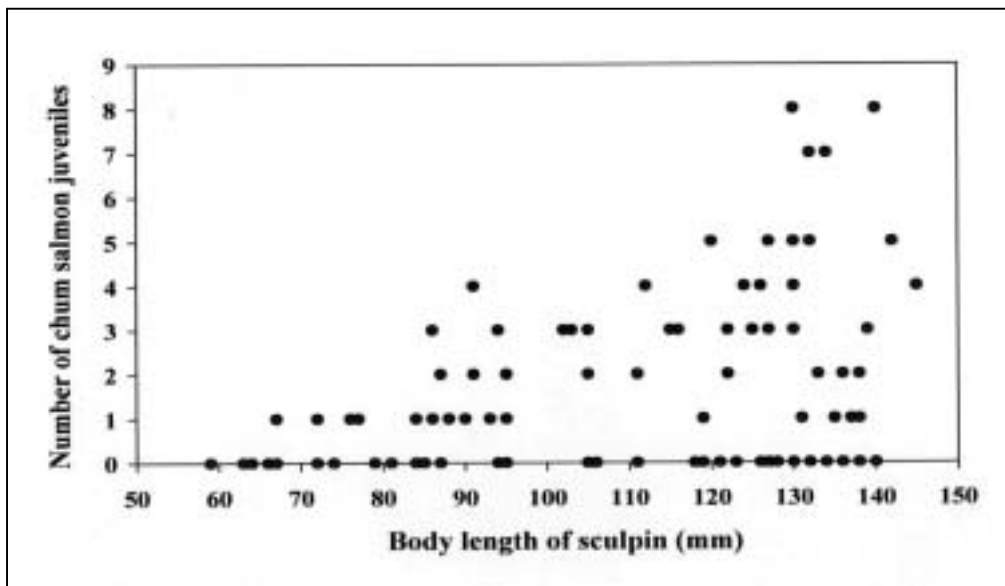

Figure 3. Relationship between number of chum salmon juveniles ingested and body length of fluvial sculpin (modified from Nagata and Miyamoto, 1986).

The total number of chum salmon consumed was calculated using equations 1 and 2, the mean BW of ingested fish and the number and mean BL of predator sculpin of each size group (with the exception of group 1). The result was estimated as 6638 fish eaten in 1983 and 6254 fish eaten in 1984 for eight days after each release. This was approximately 0.3% and 0.2% of the release number (2.51 and 3.27 million, respectively). Predation loss by the sculpin of size

group 1 was not included because they are too small (mean BL=3.25 cm) to prey on the chum salmon. The actual feeding rates of fluvial sculpin on chum salmon ranged between 27.9% and 72.2%, suggesting that the above predation rate was over-estimated. Therefore, the loss of juvenile chum salmon due to predation by fluvial sculpin appears to be minimal, making the sculpin insignificant predators in the Utabetsu River.

Predation in River-Mouth and Estuarine Areas

Nagasawa (1998) identified the Japanese dace, white-spotted charr and Japanese sea perch as predators of juvenile chum salmon in river-mouth and estuarine areas. Japanese dace are particularly abundant in these areas of northern Japan, where Kubo (1946) found them to feed before migrating to rivers. Their prey items were never identified, however. Further studies to determine their impact on chum salmon populations would be beneficial.

Recent studies have been conducted on predation by birds in these areas. In the river-mouth region of the Shokanbetsu River, western Hokkaido (Fig. 1). Kawamura *et al.* (2000) and Kawamura and Kudo (2001) observed the feeding behavior of several species of birds following a hatchery release in April 1999. The governmental hatchery is located about 7 km upstream from the mouth of the Shokanbetsu River (26 km total length). Approximately 13 million juvenile chum salmon were released in late April. Most of the fish were expected to reach the sea within 10 to 14 days post release (Kawamura, unpublished). Kawamura and the others used binoculars to count the number of gulls every 10 days from early February to late June in 1999 and observed the feeding behavior four times from late April to mid-May. They also counted the number of other birds from early April to late June. The highest count for each of the 10 days was used as a measure of the abundance of birds.

The two most frequently counted birds were black-tailed gulls *Larus crassirostris* and slaty-backed gulls *L. schistisagus.* They were followed by Japanese cormorants *Phalacrocorax filamentosus* (as *P. capillatus,* in Kawamura *et al.*, 2000), red-breasted mergansers *Mergus serrator* and harlenquin ducks *Histrionicus histrionicus* (ibid). Two more species, glaucous gulls *L. hyperboreus* and little egrets *Egretta garzetta*, were reported by Kawamura and Kudo (2001). Of these, four species (slaty-backed gulls, black-tailed gulls, red-breasted mergansers, Japanese cormorants) were found to prey on chum salmon juveniles (Kawamura *et al.*, 2000; Kawamura and Kudo, 2001).

The number of gulls counted was low in February and March but peaked in late April at over 1500 birds (Fig. 4). This period of increase directly coincided with the chum salmon release. Subsequently, the number sharply decreased in early May and remained at low levels (100-300 gulls) from mid-May to late June. Red-breasted mergansers, normally winter visitors, were most abundant (about 90 individuals) in late April. Harlequin ducks steadily decreased to about 20 from April to June, probably because this species usually migrates further north. The number of Japanese cormorants increased from April to June with a peak at 70 in early June, not corresponding with the fish release.
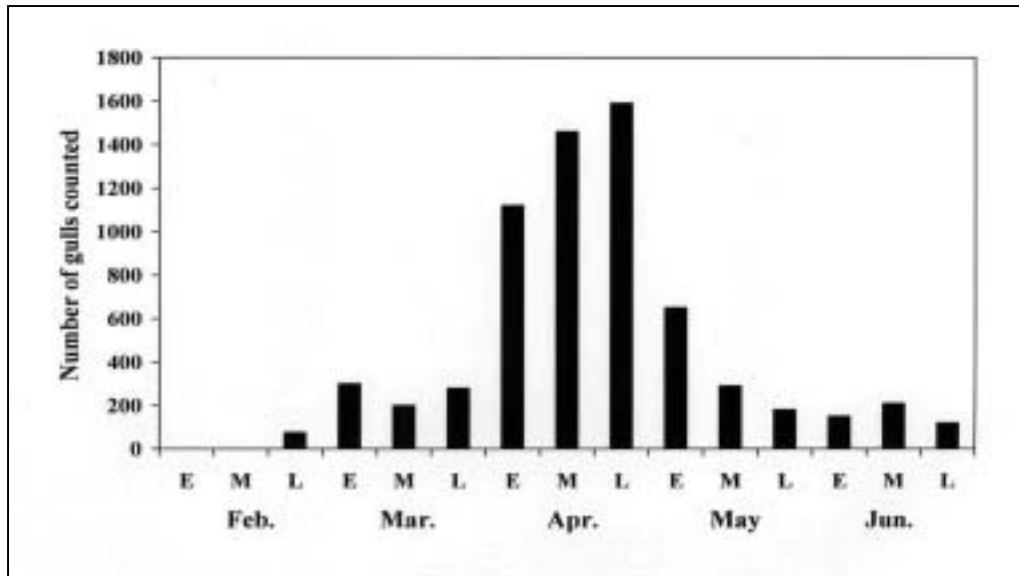
Figure 4. Seasonal changes in number of gulls counted in the mouth region of the Shokanbetsu River, western Hokkaido, from early February to late June 1999 (modified from Kawamura *et al.,* 2000). Chum salmon were released in late April, when the number of gulls peaked.

Kawamura and Kudo (2001) used data from various feeding behaviors of gulls (e.g., feeding success) and values (e.g., satiation amount of prey per day) from other studies to estimate that gulls in a 250-m region from the river mouth consumed 1.44 million chum salmon during nine days after release. This figure is equivalent to 11.1% of the number of released juveniles (13 million), indicating that the gulls are significant predators in the Shokanbetsu River. The authors stated however that the estimate was low because the survey area was restricted and other fish-eating birds were present in the region. Detailed information on estimated loss of chum salmon due to gull predation will be published elsewhere.

One of the most important factors in estimating predation impact by gulls is that these birds feed heavily daily. Although no data are available on daily food consumption of wild Japanese gulls, Harris (1965) reported that a captive herring gull *Larus argentatus* (800 g BW) consumed up to 429 g of fish in 24 hours. They are known to consume 199-368 g of fish per day in captivity (Spaans, 1971). If these figures can be applied to Japanese gulls, 400 g of fish consumed by a single gull per day, for example, would be equivalent to 476 juvenile chum salmon (mean BW=0.84 g) released into the Shokanbetsu River in 1999.

Predation in Coastal Oceanic Waters

Nagasawa (1998) reported the following fish species as predators of chum salmon in Japanese coastal waters: Far Eastern dace, Japanese halibut, spiny dogfish, arabesque greenling, pink salmon and masu salmon. Kawamura *et al.* (2000) found chum salmon juveniles in the stomachs of arabesque greenling caught in a setnet on the west coast of Hokkaido. The juveniles were not digested, so the authors suspected the arabesque greenling consumed the fish after being caught in the net. Kawamura and Kudo (2001) also examined the stomach contents of 13 fish species caught in the Sea of Japan near the mouth of the Shokanbetsu River and found that

juvenile masu salmon and white-spotted charr had eaten small chum salmon. However, the authors did not regard these salmonids as significant predators because the seaward migration period of juvenile masu salmon is different from that of chum salmon juveniles and the abundance of white-spotted charr is very low in the survey area.

Rhinoceros auklets and black-tailed gulls are major predators in coastal oceanic waters of Japan (Nagasawa and Kaeriyama, 1995; Nagasawa, 1998). Rhinoceros auklets migrate to inshore waters off western Hokkaido from late April to late May (Kawamura *et al.*, 2000). Takahashi *et al.* (2000) surveyed

Figure 5. Photograph of juvenile chum salmon with beak marks, collected in the coastal Sea of Japan on April 27, 1999 (modified from Kawamura *et al.*, 2000).

these birds in late May to July, after the coastal migration of chum salmon, on Teuri Island off western Hokkaido. They found unidentified salmonid juveniles (*Oncorhynchus* sp., annual percent occurrence of 1.1 to 3.4% in 1994-98) in the food given by adult rhinoceros auklets to their chicks. Sakurai (1984) also reported that the common tern *Sterna hirundo* feeds on chum salmon juveniles in coastal waters off eastern Hokkaido.

Along a 35-km shoreline of western Hokkaido from early April to late June in 1999, Kawamura (2001) identified 12 species of birds that peaked at approximately 3500 individuals in mid-April. Kawamura *et al.* (2000) also discovered juvenile chum salmon with beak marks on their lateral sides (Fig. 5) in late April near the Shokanbetsu River mouth, which is evidence of avian predation at sea. Based on the above information, seabirds are likely the major predators of juvenile chum salmon in the coastal sea. The most significant predators may be gulls, due to high abundance (Watanuki *et al.*, 1986; 1988) along the coast and river-mouths of western Hokkaido.

Measures to Reduce Predation Loss, and Future Research

Watanuki *et al.* (1988) reported that 36,000-40,000 pairs of black-tailed gulls and 9,600-10,000 pairs of slaty-backed gulls bred each year on the coast of Hokkaido during the 1980's. The release of chum salmon into the Shokanbetsu River attracted large numbers of gulls to the river-mouth region (Fig. 4) where the gulls were found feeding on the fish. Kawamura and Kudo (2001) estimated that gulls consumed more than ten percent of the 1999 released fish for nine days after release. Releasing hatchery-reared fish at night may reduce the gulls' ability to see them and increase fish survival.

Much remains to be studied on fish and avian predation of juvenile chum salmon in Japan. Further research is needed to assess the impact of predation by Japanese dace that abundantly occur in river-mouth and estuarine regions of northern Japan and to study the feeding ecology of gulls in river-mouth regions and coastal oceanic waters. Research is also needed to clarify the anti-predator behavior of released juvenile chum salmon and to develop measures to reduce predation. In Honshu, along the Japan Sea coast, both chum and masu salmon are

frequently released into the same rivers. Since masu salmon are known to feed on chum salmon (Tago, 1994), investigations to estimate predation loss may show the need to identify different locations for release.

Acknowledgments

Literature Cited

Amida, K. and M. Okada. 1973. Studies on mortality factors of chum salmon-I. Habits of the folktongue goby as predator of chum salmon juveniles. *Aquaculture* 21:62-64 (in Japanese).

Beamish, R. J., B. L. Thomson, and G. A. Mcfarlane. 1992. Spiny dogfish predation on chinook and coho salmon and the potential effects on hatchery-produced salmon. *Trans. Am. Fish. Soc*. 121:444-455.

Collis, K., D. D. Roby, D. P. Craig, B. A. Ryan, and R. D. Ledgerwood. 2001. Colonial waterbird predation on juvenile salmonids tagged with passive integrated transponders in the Columbia River estuary: vulnerability of different salmonids species, stocks, and rearing types. *Trans. Am. Fish. Soc.* 130:385-396.

Goto, A. 1989. *Cottus nozawae*. In: H. Kawanabe, and N. Mizuno, (eds.), Freshwater Fishes of Japan, Yama-Kei Publ., Tokyo, p. 660-665 (in Japanese).

Harris, M. P. 1965. The food of some *Larus* gulls. *Ibis* 107:43-52.

Hikita, T. and A. Nagasawa. 1960. Biological observations of Memu stream, Tokachi River system. The damage of salmon eggs and fry by predaceous fishes. *Sci. Rep. Hokkaido Salmon Hatchery* 15:69-83 (in Japanese with English abstract).

Hikita, T., S. Kameyama, A. Kobayashi, and Y. Sato. 1959. Some biological observations on the rainbow trout, *Salmo gairdneri irideus*, in the Nishibetsu River, with special reference to the consumption of salmon eggs and fry by predaceous fishes. *Sci. Rep. Hokkaido Salmon Hatchery* 14:91-121 (in Japanese with English abstract).

Hiyama, Y., Y. Nose, M. Shimizu, J. Matsubara, K. Onodera, Y. Kodama, and N. Suzuki. 1960. Predation of rainbow trout fry traced by radio-isotope. *Bull. Japan. Soc. Sci. Fish.* 26:863-867 (in Japanese with English abstract).

Hiyama, Y., Y. Nose, M. Shimizu, T. Ishihara, H. Abe, R. Sato, and T. Maiwa. 1972a. Predation of chum salmon fry during the course of its seaward migration-I. Otsuchi River investigation 1961-1963. *Bull. Japan. Soc. Sci. Fish*. 38:211-221.

Hiyama, Y., Y. Nose, M. Shimizu, T. Ishihara, H. Abe, R. Sato, T. Maiwa, and T. Kajihara. 1972b. Predation of chum salmon fry during the course of its seaward migration-II. Otsuchi River investigation 1964 and 1965. *Bull. Japan. Soc. Sci. Fish.* 38:223-229.

Inukai, T. 1949. Feeding habit of Japanese dace in the Bibi River, Hokkaido. *Sci. Rep. Hokkaido Fish Hatchery* 4:57-61 (in Japanese).

Irie, T. 1990. Ecological studies on the migration of juvenile chum salmon, *Oncorhynchus keta*, during early ocean life. *Bull. Seikai Nat. Fish. Res. Inst*. 68:1-142 (in Japanese with English abstract).

Kaeriyama, M. 2000. Hatchery programmes and stock management of salmonids populations in Japan. In: B. R. Howell, E. Moksness, and T. SvΔsand, (eds.), Stock Enhancement and Sea Ranching, Fishing News Books, Oxford, p. 153-167.

Kawamura, H. 1979. Distribution, movement and age constitution of the common freshwater sculpin (*Cottus pollux* Günther) in the salmon propagation river. *Sci. Rep. Hokkaido Fish Hatchery* 34:17-24 (in Japanese with English abstract).

Kawamura, H. 1980. Predation of chum salmon fry by common freshwater sculpins (*Cottus pollux* Günther) in the salmon propagation river. *Sci. Rep. Hokkaido Fish Hatchery* 35:53-62 (in Japanese with English abstract).

Kawamura, H. 2001. Coastal ecology and survival of chum salmon juveniles. Uo to Mizu 37:25-30 (in Japanese).

Kawamura, H. and S. Kudo. 2001. Seabird predation on juvenile chum salmon. NPAFC Tech. Rep. 2:9-10.

Kawamura, H., S. Kudo, M. Miyamoto, M. Nagata, and K. Hirano. 2000. Movements, food and predators of juvenile chum salmon (*Oncorhynchus keta*) entering the coastal Sea of Japan off northern Hokkaido in warm and cool years. *N. Pac. Anadr. Fish Comm. Bull.* 2:23-41.

Kubo, T. 1946. On the predation on salmonid juveniles by various fishes in rivers. *Sci. Rep. Hokkaido Fish Hatchery* 1:51-55 (in Japanese).

Nagasawa, K. 1998. Fish and seabird predation on juvenile chum salmon (*Oncorhynchus keta*) in Japanese coastal waters, and an evaluation of the impact. *N. Pac. Anadr. Fish Comm. Bull.* 1:480-495.

Nagasawa, K. and M. Kaeriyama. 1995. Predation by fishes and seabirds on juvenile chum salmon (*Oncorhynchus keta*) in coastal waters of Japan: a review. *Sci. Rep. Hokkaido Salmon Hatchery* 49:41-53 (in Japanese with English abstract).

Nagata, M. 1984. Ecological studies on the predation of chum salmon fry by fresh water sculpin, *Cottus nozawae* Synder. *Sci. Rep. Hokkaido Fish Hatchery* 39:55-65 (in Japanese with English abstract).

Nagata, M. and M. Miyamoto. 1986. The downstream migration of chum salmon fry, *Oncorhynchus keta*, released into the Utabetsu River of eastern Hidaka in Hokkaido, and the estimation of predation amount of the fry by fresh water sculpin, *Cottus nozawae* Synder. *Sci. Rep. Hokkaido Fish Hatchery* 41:13-22 (in Japanese with English abstract).

Nakano, S. 1992. A preliminary report on food habit of Japanese huchen *Hucho perryi* in northern Hokkaido. *Biol. Inl. Wat.* 7:20-23 (in Japanese with English abstract).

Rieman, B. E., R. C. Beamesderfer, S. Vigg, and T. P. Poe. 1991. Estimated loss of juvenile salmonids to predation by northern squawfish, walleyes, and smallmouth bass in John Day Reservoir, Columbia River. *Trans. Am. Fish. Soc.* 120:448-458.

Ruggerone, G. T. 1986. Consumption of migrating juvenile salmonids by gulls foraging below a Columbia River dam. *Trans. Am. Fish. Soc.* 115:736-742.

Ruggerone, G. T. and D. E. Rogers. 1992. Predation on sockeye salmon fry by juvenile coho salmon in the Chignik Lakes, Alaska: implications for salmon management. *North Am. J. Fish. Management* 12:87-102.

Sakurai, A. 1984. Chum salmon. Heibonsha, Tokyo, 51 p. (in Japanese).

Spaans, A. L. 1971. On the feeding ecology of the herring gull *Larus argentatus* in the northern part of the Netherlands. *Ardea* 59:73-188.

Tago, Y. 1994. Feeding habit of juvenile masu salmon, *Oncorhynchus masou*, during the period of seaward migration in the Shou River of Toyama Prefecture. *Bull. Toyama Pref. Fish. Exp. Stn*. 5:13-20 (in Japanese with English abstract).

Takahashi, A., M. Kuroki, Y. Niizuma, A. Kato, S. Saito, and Y. Watanuki. 2001. Importance of the Japanese anchovy (*Engraulis japonicus*) to breeding rhinoceros auklets (*Cerorhinca monocerata*) on Teuri Island, Sea of Japan. *Mar. Ecol.* 139:361-371.

Takami, T. and K. Nagasawa. 1996. Predation on chum salmon (*Oncorhynchus keta*) fry and masu salmon (*O. masou*) juveniles by white-spotted charr (*Salvelinus leucomaenis*) in a river in northern Japan. *Sci. Rep. Hokkaido Fish Hatchery* 50:45-47.

Watanuki, Y., M. Aotsuka, and T. Terasawa. 1986. Status of seabirds breeding on Teuri Island. *Tori*   34:146-150 (in Japanese with English abstract).

Watanuki, Y., N. Kondo, and H. Nakagawa. 1988. Status of seabirds breeding in Hokkaido. *Jap. J. Ornithol*. 37:17-32 (in Japanese with English abstract).

Wood, C. C. 1987. Predation of juvenile Pacific salmon by the common merganser (*Mergus merganser*) on eastern Vancouver Island. I: Predation during the seaward migration. *Can. J. Fish. Aquat. Sci.* 44:941-949.

Interaction Between Cleaner and Host: The Black Porgy Cleaning Behavior of Juvenile Sharpnose Tigerfish, *Rhyncopelates oxyrhynchus* in the Seto Inland Sea, Western Japan

Toshihiro Shigeta
Hironori Usuki
National Research Institute of Fisheries and Environment of Inland Sea (F.E.I.S.)
2-17-5 Maruishi, Oono
Hiroshima 739-0452
JAPAN
Email: shigeta@fra.affrc.go.jp

Kenji Gushima
Faculty of Applied Biological Science
Hiroshima University
1-4-4 Kagamiyama, Higashi-Hiroshima
Hiroshima 739-8528
JAPAN

Abstract

The cleaning behavior of a juvenile sharpnose tigerfish, *Rhyncopelates oxyrhynchus* was first observed in the fishing port of Hiroshima Bay in the Seto Inland Sea, Japan. In the port, only five fishes including the black porgy *Acanthopagrus schlegeli* recognized the cleaner and posed. Among these, four were inspected and picked by the cleaner. From both the cleaner and the host respects, the cleaning behavior was divided into three parts: the start of cleaning, the formation of a shoal and the termination of cleaning. From the host side, before the start, the host's soliciting behavior consisted of four parts; detection, recognition, approach and the pose to the cleaner. From the cleaner's side, before the start, the cleaner inspected a host on its body surface.

In particular, a series of black porgy soliciting behavior was investigated in detail. It consisted of four consecutive parts. The host aggregated 0.5-0.8 m apart. The aggregated black porgies were each 10-50 cm in total length (TL). The cleaners were 10 cm $\pm$ 1 SD in TL. No signal behavior from the cleaner for its host to start cleaning was observed. After the cleaner had picked against the host several times, it moved serially to other individuals. Cleaners were not observed to clean hosts smaller than themselves. Cleaning stations were formed in several places located along cleaner foraging migration routes. They were located 0.7 m $\pm$ 0.5 SD in depth on sand-mud or artificial concrete bottoms.

At least thirty-four cleaner fishes, including *R. oxyrhynchus*, were noted as living in Japan.

Introduction

Cleaning symbiosis is a well-known phenomenon among marine fishes (Losey *et al.*, 1999). The cleaners feed on ectoparasites and other material from the body surface of cooperating hosts. During cleaning the host displays a stationary pose. It is assumed that cleaning behavior exists in almost all aquatic environments (Helfman *et al.,* 1997). There has been little study on the behavior

of fishes in the sea around Japan, except for the cleaning wrasse *Labroides dimidiatus* which is often used to control ectoparasites on fish exhibited in aquariums (Kuwamura, 1976; Chikasue, unpublished data [1]; Ueharako, unpublished data [2]).

In July 2000, in a fishing port in the Seto Inland Sea of Western Japan, we found that a juvenile sharpnose tigerfish, *Rhyncopelates oxyrhynchus* (Terapontidae) performed cleaning-like behavior with large-sized, black porgies, *Acanthopagrus schlegeli* (Sparidae). Along with a brief review of cleaning fishes in Japan, we report in detail the newly discovered cleaning relationship between the above two fishes.

Materials and Methods

During the daytime from July to October 2000, we observed the behavior of *R. oxyrhynchus* in a fishing port in Hiroshima Bay. The study site was situated in the western part of the Seto Inland Sea (Fig.1). The port is approximately 40,000 m^2 with the deepest place being 5 m, in the center. Except in the center, most areas are exposed to the air at ebb tide.

We observed the tigerfish behavior with host fishes, especially the black porgy, one of the most important fishery and aquaculture species in Japan. We estimated the total length (TL) of the cleaners and hosts using ruler calibration by 0.5 cm size classes for fish less than 15 cm TL or 1 cm size classes for fish 15 cm TL and over. We defined the approaching distance of hosts to cleaners as the distance between the point where a host began to approach

Figure 1. Map shows Hiroshima Bay in the Seto Inland Sea, Japan. The star mark indicates our study site in the fishing port near the National Research Institute of Fisheries and Environment of Inland Sea (F.E.I.S.).

a cleaner and the point where the cleaner was located. We recorded the water depth and benthic phase on the cleaning station. The distance and the depth were estimated to the nearest 5 cm.
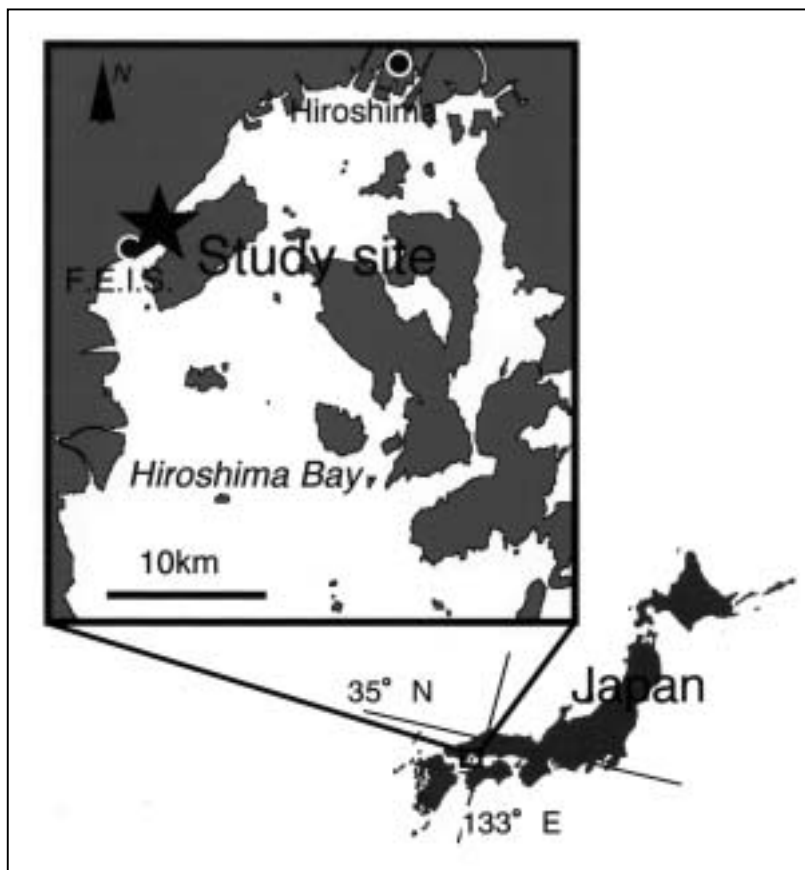
1 **Chikasue, M.** 1989. The influence of ectoparasites on the cleaning symbiosis between a wrasse, *Labroides dimidiatus* (Labridae), and its host fishes. M.Sc. thesis, Ehime University, Ehime, JAPAN, 22 p.

2 **Ueharako, A.** 2000. The influence of ectoparasites on host fishes' behavior. M.Sc. thesis, Ehime University, Ehime, JAPAN, 26 p. (In Japanese).

The classification and scientific names of fishes followed Nakabo (2000) except *Halichoeres bleekeri* and *Coris musume*. *Halichoeres bleekeri* and *C. musume* followed Randall (1999a) and Randall (1999b), respectively.

Results

More than 26 species ranging from Clupeidae to Tetraodontidae were recorded in the fishing port (Shigeta, unpublished data). Only five of these recognized the tigerfish as a cleaner and posed with a species-specific figure against the cleaner (Table 1). The five belonged to Mugiliformes and Perciformes. All but *A. latus* were inspected and picked by the cleaner (Table 2). Three hosts, the black porgy and two mullet species, were observed many times cleaning for long periods.

Table 1. List of fishes that displayed a pose for the cleaner *R. oxyrhyncus* in the fishing port.

Order	Family	Species
Mugiliformes	Mugilidae	*Mugil cephalus cephalus*
		Chelon haematocheilus
Perciformes	Sparidae	*Acanthopagrus schlegeli*
		Acanthopagrus latus[1]
	Terapontidae	*Rhyncopelates oxyrhynchus*

1: Only one individual of this species was found and its pose was observed only once.

Table 2. List of fishes that received cleaning from *R. oxyrhyncus* in the fishing port.

Order	Family	Species
Mugiliformes	Mugilidae	*Mugil cephalus cephalus*
		Chelon haematocheilus
Perciformes	Sparidae	*Acanthopagrus schlegeli*
	Terapontidae	*Rhyncopelates oxyrhynchus*

Cleaning behavior was divided into three parts: the start of cleaning, the formation of a shoal and the termination of cleaning (Fig. 2). Before the beginning of cleaning, hosts solicited the cleaner. This behavior generally consisted of four parts: detection of a cleaner, recognition, approach and pose. Before the start of cleaning, the cleaner inspected the body surface of the posing host.
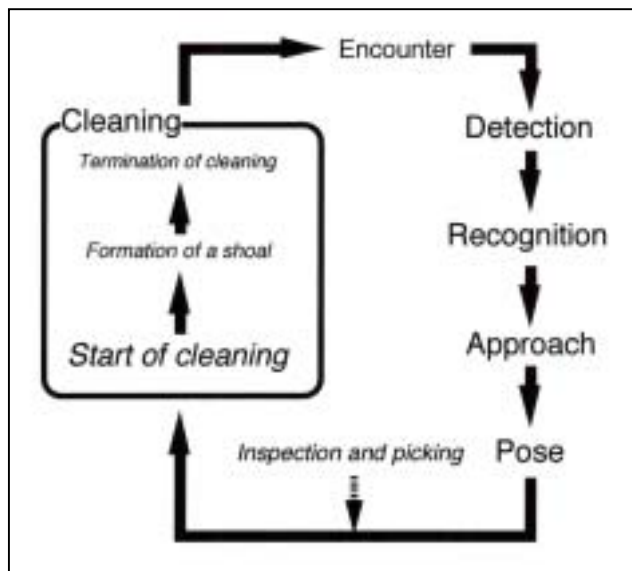
Figure 2. General process of the cleaning behavior of *R. oxyrhynchus* from the view of its host. Each item indicates the host's performance mainly. Italic items in the box show the three cleaning periods. The italic item with the broken arrow shows the cleaner's behavior.

Black porgy (10-50 cm TL) soliciting behaviors consisted of four movements. When a host detected a cleaner, the host that wished cleaning rapidly changed its direction toward the potential cleaner. The host aggregated from 0.5-0.8 m apart (n=3). That is, the approaching distance was 2.2-5.7 times the host's TL. Next, the host moved close to the cleaner and prevented the cleaner from bottom feeding by closely intruding in front of the cleaner's head. The host then showed its lateral side to the cleaner and assumed a slight head-down position, spreading wide its bilateral pectoral, pelvic, anal, and occasionally dorsal fins, while hovering motionlessly (Fig. 3). Blanching in color or opening of the mouth or gill covers was not observed in any host. In a shoal some hosts seemed to lose control and gradually revealed a curious vertical posture (Fig. 3).

As a result of solicitation by the black porgy, the tigerfish stopped foraging on the bottom. After leaving the bottom, as a true cleaner, it began to inspect the surface of the host and to clean. These bouts were the beginning of cleaning behavior. Total lengths of the cleaners were 10 cm $\pm$1 SD (range 6-12, n = 20). No signal behavior of the cleaner for its host to start cleaning was observed. The tigerfish inspected the host's body surface carefully, and then picked on several parts. The cleaner especially preferred near the top of the head, and the base and upper parts of the

Figure 3. Cleaner *R. oxyrhynchus* (A, 10 cm TL) is inspecting the body surface of a black porgy, *A. schlegeli,* and picking off ectoparasites. Each host, *A. schlegeli,* displays a stationary pose. Among them, the central largest porgy loses its balance and shows a vertical posture (B).

caudal fin. However, cleaning into the host's mouth or its gill cover was not observed. After the cleaner picked against the host several times, it moved to other individuals. No cleaner chose a host smaller than itself (n = 17).
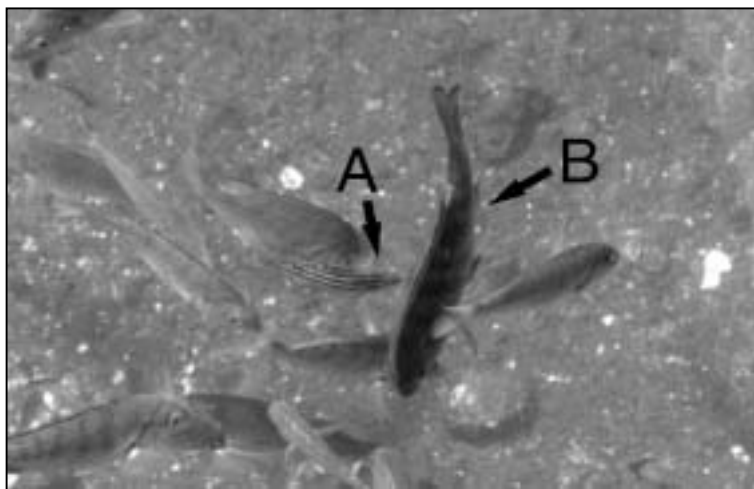
All porgies aggregated prior to cleaning, forming a shoal of porgies (Fig. 4). Cleaning stations formed in several places located along consistent foraging migration routes. These stations were located 0.7 m $\pm$ 0.5 SD (range 0.3-2.0, n=6) in depth on sand-mud or concrete bottoms, in weak current areas, such as points where boats were moored, spaces behind large pipes and in the step hollows of a concrete seawall.
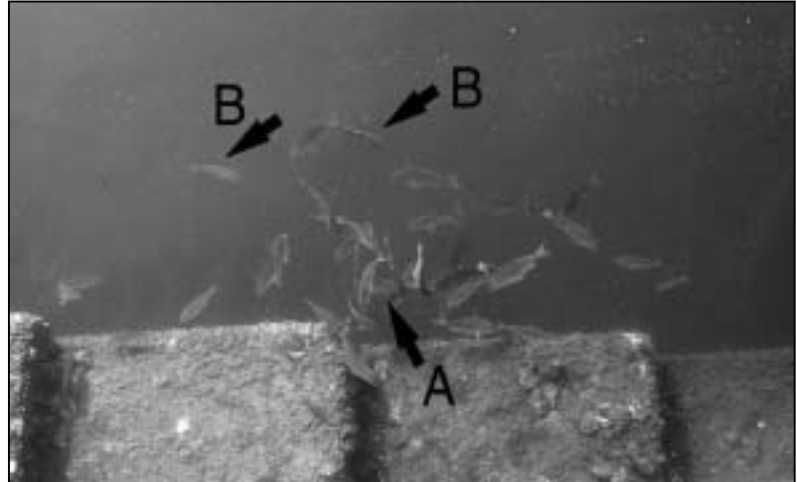

Figure 4. A shoal of cleaning black porgies (12-38 cm TL) near steps on the seawall. The two gray mullet *Mugil cephalus cephalus,* 25 cm TL (B), can never intrude into the center of the shoal because of interspecies hierarchy. Only one cleaner, the same individual as shown in Fig. 3, exists in the center (A).

Discussion

It is assumed that cleaning behavior exists in almost all aquatic environments. Thirty-four cleaner species, including *R. oxyrhynchus,* are noted as living in Japan (Table 3). However, there have been no detailed Japanese reports on their behavior, except for *L. dimidiatus.* Among these thirty-four species, *R. oxyrhynchus, Psedolabrus japonicus,* and *H. bleekeri* are common in the Seto Inland Sea, a typical temperate area of Japan.

Table 3. List of cleaning fishes in Japan.

Order	Family	Species	Source
Siluriformes	Plotosidae	*Plotosus lineatus*	Okata (1994)
Gasterosteiformes	Syngnathidae	*Doryrhamphus excisus excisus*	Myers (1989)
		Doryrhamphus japonicus	Yanagita (1990)
Perciformes	Echeneidae	*Echeneis naucrates*	Cressey and Lachner (1970)
		Phtheirichthys lineatus	Cressey and Lachner (1970)
		Remora remora	Cressey and Lachner (1970)
		Remora osteochir	Cressey and Lachner (1970)
		Remora brachyptera	Strasburg (1959)
		Remora pallida	Strasburg (1959)
	Chaetodontidae	*Heniochus monoceros*[1]	Shigeta (unpubl. data)
		Heniochus acuminatus[1]	Shigeta (unpubl. data)

Table 3, Continued

Order	Family	Species	Source
		Heniochus diphreutes	Randall (1985)
		Chaetodon plebeius[2]	Sadovy and Cornish (2000)
	Pomacanthidae	*Pomacanthus imperator*	Hirata *et al.* (1996)
	Terapontidae	*Rhyncopelates oxyrhynchus*	Our present study.
	Labridae	*Pseudodax moluccanus*	Randall (1992)
		Bodianus axillaris	Randall (1992)
		Bodianus diana	Randall (1992)
		Labroides dimidiatus	Randall (1958)
		Labroides bicolor	Randall (1958)
		Labroides pectoralis	Randall and Springer (1975)
		Labroides rubrolabiatus	Randall (1958)
		Labrichthys unilineatus	Debelius (1993)
		Labropsis manabei	Masuda and Kobayashi (1994)
		Labropsis xanthonota	Randall (1981)
		Pseudolabrus japonicus[3]	Chikasue (unpubl. data) [4]
		Thalassoma cupido	Kuwamura (1976)
		Thalassoma amblycephalum	Debelius (1993)
		Thalassoma lunare	Okata (1994)
		Halichoeres bleekeri[5]	Chikasue (unpubl. data) [4]
		Coris musume	Hirata *et al.* (1996)
	Acanthuridae	*Prionurus scalprum*	Kuwamura (1976)
Pleuronectiformes	Pleuronectidae	*Pleuronectes schrenki*	Ho *et al.* (2001)
Tetraodontiformes	Ostraciidae	*Ostracion immaculatus[1]*	Shigeta (unpubl. data)

1: Observation of cleaning behavior in an aquarium.

2: Observation in captivity.

3: *P. japanicus* is divided into two species, *P. sieboldi* and *P. eoethinus*, by Mabuchi and Nakabo (1997). Only *P. sieboldi* inhabits the Seto Inland Sea. However, we do not differentiate these in this paper, a review would be necessary .

4: See text's footnote 1 shown before.

5: *H. tenuispinis* is divided into two species, *H. tenuispinis* and *H. bleekeri*, by Randall (1999 a). Only *H. bleekeri* inhabits Japan. On the basis of the report, the fish, *H. tenuispinis* which Chikasue observed is *H. bleekeri*.

Most cleaner species maintain conspicuous color patterns on their lateral sides, such as stripes on the body or fins. Juvenile *R. oxyrhynchus* have a yellowish, silvery body color with four clear black

longitudinal stripes. More than twenty-six fish species living in the fishing port, except *H. poecilopterus*, usually have sober color patterns. According to Hidaka (1998) the tigerfish dorsal color pattern may serve as a camouflage from birds, while the lateral pattern may advertise itself as a cleaner.

Only five fishes living in the port were observed to pose in cleaning behavior. The result may show that there are fish that do not recognize *R. oxyrhynchus* as a cleaner. For example, although the numbers in the port of large *Tribolodon hakonensis* equal those of black porgy or mullet, this fish never takes an interest in cleaning.

After a host's recognition, the approach and pose are conducted. This behavior may be the result of the host's desire to rid itself of ectoparasites. The soliciting behavior of black porgies for cleaning indicates the high intention to clean. If a tigerfish removed scales or a piece of the fins from the black porgy's body, the porgy would more than likely avoid it. Past studies on the proximate causes of posing against *Labroides* spp. have suggested that the hosts are attracted to the cleaner wrasse to obtain gentle tactile stimulation (Losey, 1971; Losey and Margules; 1974; Losey, 1979). However, recent studies of cleaning wrasses have shown that parasitism on the host is positively correlated to the frequency of the pose (Chikasue, unpublished data [1]; Ueharako, unpublished data [2]; Grutter, 2001). In all cases, the eagerness of a host for cleaning is essential for the series of cleaning behavior.

The sharpnose tigerfish obviously performed cleaning behavior. Its diet includes not only small benthic invertebrates such as copepods, ostracods and gammarids in the substrate, but also caligid copepods that usually inhabit the body surface of fish (our unpublished data). The feeding habits also strongly support cleaning behavior. It is necessary to analyze the feeding habits to see if the cleaner is picking off anything other than ectoparasites. In certain conditions, this opportunistic predator may change its feeding habitat from the bottom to the surface of the host body. The biomass of both the benthic animals and the ectoparasites on the host needs to be investigated in detail to clarify this switching mechanism.

European sea lice are parasites on Atlantic salmon that cause serious problems in aquaculture (MacKinnon, 1997). European wrasses are used to help reduce ectoparasite infestations on salmon kept in aquaculture pens (Sayer *et al.*, 1996). Ectoparasites also cause substantial damage to aquaculture in Japan. For the extermination of these ectoparasites, medicated or fresh-water bath methods have been adopted. However, the former causes environmental pollution around aquaculture pens, whereas the latter involves extensive handling. The utilization of the symbiotic cleaning relationship detailed here may resolve those problems, and may in the future help attain an environmentally benign aquaculture system for Japan. Further research on this phenomenon would clarify this possibility and its application in aquaculture.

Acknowledgments

Literature Cited

Cressey, R.F., and E.A. Lachner. 1970. The parasitic copepod diet and life history of disk fishes (Echeneidae). *Copeia*. 2:310-318.

Debelius, H. 1993. Indian ocean tropical fish guide. IKAN-Unterwasserarchiv, Frankfurt, Germany, 321 p.

Grutter, A.S. 2001. Parasite infection rather than tactile stimulation is the proximate cause of cleaning behavior in reef fish. *Proc. Roy. Soc. Lond.* 268:1361-1365.

Helfman, G.S., B.B. Collette, and D.E. Facey. 1997. The Diversity of Fishes. Blackwell Science, Oxford, UK, xii+528 p.

Hidaka, T. 1998. Colors and patterns of the fishes: What is their function? *Aquabiology* 119:451-456 (In Japanese with English abstract).

Hirata, T., T. Yamakawa, A. Iwata, S. Manabe, W. Hiramatsu, and N. Ohnishi. 1996. Fish fauna of Kashiwa-jima Island, Kochi Prefecture, Japan. *Bull. Mar. Sci. Fish.*, *Kochi Univ.,* 16:1-177 (In Japanese with English abstract).

Ho, J-S., K. Nagasawa, and T. Takatsu. 2001. The juvenile Cresthead flounder (*Pleuronectes schrenki*): an occasional cleaner occurring in Lake Notoro, Hokkaido. *Bull. Fish. Sci. Hokkaido Univ.* 52(1):1-3.

Kuwamura, T. 1976. Different responses of inshore fishes to the cleaning wrasse, *Labroides dimidiatus,* as observed in Sirahama. *Publ. Seto Mar. Biol. Lab*. 23:119-144.

Losey, G.S. 1971. Communication between fishes in cleaning symbiosis. In: Aspects of the biology of symbiosis. C. Cheng (ed.), p. 45-76. Univ. Park Press, Baltimore, MD, USA.

Losey, G.S. and L. Margules. 1974. Cleaning symbiosis provides a positive reinforcement for fish. *Science* 184:179-180.

Losey, G.S. 1979. Fish cleaning symbiosis: proximate causes of host behavior. *Anim . Behav*. 27:669-685.

Losey, G.S., A.S. Grutter, G. Rosenquist, J.L. Mahon, and J.P. Zamzow. 1999. Cleaning symbiosis: a review. In: Behaviour and Conservation of Littoral Fishes, V.C. Almada, R.F. Oliveira and E.J. Goncalves (eds.), p. 379-395. Instuto Superior de Psichologia Aplicada, Lisbon, Portugal.

Mabuchi, K. and T. Nakabo. 1997. Revision of the genus *Pseudolabrus* (Labridae) from the East Asian Waters. *Ichthyol. Res*. 44(4):321-334.

MacKinnon, B.M. 1997. Sea Lice: a review. *World Aquaculture*, September, p. 5-10.

Masuda, H. and Y. Kobayashi. 1994. Grand Atlas of Fish Life Modes, Tokai Univ. Press, Tokyo, Japan, 47+465 p. (In Japanese).

Myers, R.R. 1989. Micronesian reef fishes. Coral Graphics, Barrigada, Guam, USA, vi+298 p.

Nakabo T. (ed). 2000. Fishes of Japan with pictorial keys to the species, 2nd ed., Tokai Univ. Press, Tokyo, Japan, lvi+1748 p. (In Japanese).

Okata, Y. 1994. The world of fishes: my field notes of underwater observation. Kaiyuusya, Tokyo, Japan, 421+13 p. (In Japanese).

Randall, J.E. 1958. A review of the labrid fish genus *Labroides*, with descriptions of two new species and notes on ecology. *Pac. Sci.* 12:327-347.

Randall, J.E. 1981. Revision of the Labrid fish genus *Labropsis* with descriptions of five new species. *Micronesica* 17(1-2):125-155.

Randall, J.E. 1985. Guide to Hawaiian reef fishes. Harrowood Books, Newtown Square, PA, USA, 74 p.+177 pl.

Randall, J.E. 1992. Diver's guide to fishes of Maldives. Immel Publishing, London, UK, 193 p.

Randall, J.E. 1999 a. *Halichoeres bleekeri* (Steindachner & Döderlein), a valid Japanese species of labrid fish, distinct from *H. tenuispinis* (Günther) from China. *Ichthyol. Res.* 46(3):225-231.

Randall, J.E. 1999 b. Revision of Indo-Pacific Labrid fishes of the genus *Coris*, with description of five new species. *Indo-Pacific fishes* 29:1-74.

Randall, J.E., and V.G. Springer 1975. *Labroides pectoralis*, a new species of Labrid fish from the tropical western Pacific. *Uo* 25:4-11.

Sadovy, Y., and A.S. Cornish 2000. Reef fishes of Hong Kong. Hong Kong Univ. Press, Aberdeen, Hong Kong, China, xi+321 p.

Sayer, M.D., J.W. Treasurer, and M.J. Costello (eds.). 1996. Wrasse: biology and use in aquaculture. Fishing News Books, London, UK, ix+283 p.

Strasburg, D.W. 1959. Notes on the diet and correlating structures of some central Pacific echeneid fishes. *Copeia* 3:244-248.

Yanagita, M. 1990. The fishes inhabiting the inlet in Okuridashi. *I.O.P. Diving News* 1(8):2-3. (In Japanese).

■ IV. CASE STUDIES

Alaska Salmon Enhancement: A Successful Program for Hatchery and Wild Stocks

William R. Heard
NOAA/NMFS-Auke Bay Laboratory
Alaska Fisheries Science Center
11305 Glacier Highway
Juneau, Alaska 99801
USA
Email: bill.heard@noaa.gov

Abstract

Alaska salmon have been the focus of major commercial harvesting since the latter part of the 1800s. Cyclic fluctuations of salmon abundance in various regions of the state, sometimes synchronous statewide, cause periods of high or low run strength associated with variable harvest levels. Poor harvest from weak wild stocks can result in socio-economic disruptions throughout the state. Modern salmon hatcheries in Alaska were developed in response to record low wild-stock runs in the 1960s and 1970s and now provide important complements to fisheries dependent on these resources. Initially conceived as state-run systems, most Alaskan hatcheries are now run by private sector corporations, primarily regional aquaculture associations comprised of fishermen and other stakeholders. Alaska now has 33 production hatcheries in a balanced program designed to enhance fisheries while maintaining healthy wild stocks. Some hatcheries release over 100 million juvenile salmon annually. Statewide totals are 1.2 to 1.4 billion annually over the last decade. Following a major turnaround from record low runs two decades earlier, commercial harvest of Alaska salmon in the 1990s have been at or near record high levels, although wild stocks in some areas, especially western Alaska, are at depressed levels. During the past decade hatcheries have produced 27-63 million adults annually, accounting for 14-37% of common-property statewide commercial harvest. This successful hatchery program, however, involves some controversy and some scientists have challenged its effectiveness. As Alaskan salmon enhancement has matured 13 hatcheries have been closed for various reasons. This salmon enhancement program has focused on protecting wild stock fitness and other unique stock characteristics through strict regulation by geneticists, pathologists, and managers for siting and capacity of hatcheries. The use of hatchery brood stocks has been restricted and controlled by policy and regulation. New mass-marking technologies have enabled harvest managers to better protect wild stocks in mixed-stock fisheries. A cornerstone of Alaska's successful salmon program has been the application of escapement-based, adaptive management for wild stocks and judicious use of technologies appropriate for enhancement. Salmon enhancement in Alaska is designed to supplement common property fisheries, not to supplement wild spawning populations or to rebuild depressed wild stocks with hatchery-origin fish. In spite of generally healthy wild stocks with important contributions from hatcheries, the economics of Alaska's commercial salmon industry, based on common property capture fisheries, is clouded by competition from continuing increases in worldwide production of farmed salmon. As global production annually, approaches 2 million tons farmed production now surpasses all capture fisheries for salmon.

Salmon runs in Alaska may be characterized by cyclic periods of high and low abundance that may last for decades. These cycles are varied and generally not well understood. The dramatic shift in numbers of returning fish, once thought to be primarily caused by harvest levels, spawning escapements, and various survival factors in freshwater habitats, are now known to be affected by cyclic, climatic and environmental fluctuations during the marine life history of salmon (Francis and Hare, 1994; Mantua *et al.*,1997).

Alaska commercial salmon fisheries have a colorful history spanning a 120-year period (Byerly *et al.*, 1999). After reaching record high harvest levels of over 100 million salmon caught annually during the 1940's, a long decline began to reduce annual catch levels to 50 million by the 1960s. This was shortly after Alaska had attained statehood and some felt a long period of pre-statehood federal mismanagement, highlighted by the widespread use of fish traps, was responsible for the decline. Runs began to recover briefly under management by the new State of Alaska only to take a new downturn that resulted in only 22 million salmon caught commercially in 1973 (Fig. 1).
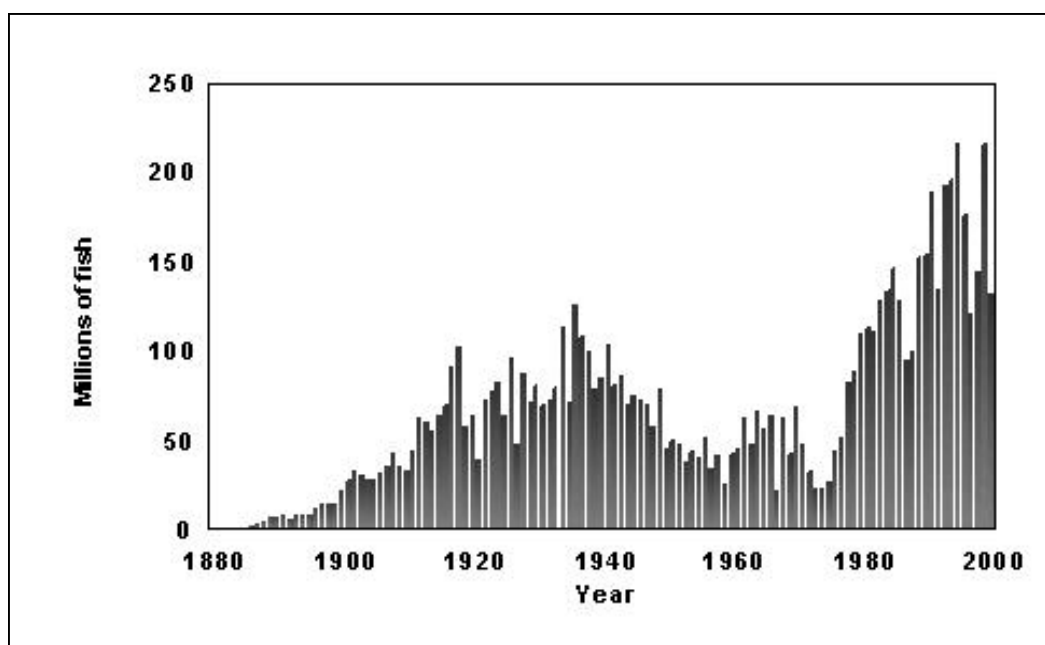

Figure 1. Commercial salmon catches in Alaska, 1880s-2000.

In response to these low returns, the Alaska State Legislature in 1971 created a new division within the Alaska Department of Fish and Game (ADF&G) to develop a coordinated salmon enhancement program. This was soon followed by legislation authorizing the concept of private non-profit hatcheries and the formation of regional aquaculture associations. Primary responsibilities and goals of this new legislation were to: [AS 16.05.092] (1) develop a comprehensive, coordinated state plan for the orderly rehabilitation, enhancement, and development of the state's fisheries; (2) encourage investment by private enterprise in technological development and economic utilization of fisheries resources; and (3) encourage, sponsor, and conduct research on the basic problems limiting sound development of hatcheries.

Over the next several years, as the new focus on salmon enhancement was implemented, a dramatic turnaround occurred in overall abundance and run strength of Alaska's wild stocks. This improvement in wild stocks, strongly influenced by better ocean conditions due to climatic regime shifts (Hare and Mantua, 2000), was concomitant with favorable performance levels of most new hatcheries as they came on line. By the 1990s commercial salmon harvests were at or near record levels in many regions of the state. On a statewide basis commercial harvests since 1980 have exceeded 100 million salmon every year except in 1987. Record harvests of 217 million and 216 million salmon occurred in 1995 and 1999, respectively (Fig. 1). As wild stocks produced impressive catches during the 1990s, hatcheries also produced 27-63 million adults annually, accounting for 14-37% of statewide common-property harvest (McNair, 1999, 2001).

In this document I review elements of current Alaska salmon hatchery programs with a focus on the development of policies guiding their operation and performance. I also discuss the general status of wild stocks, fisheries, economics, and controversies in different regions of the state.

Policies and Procedures

Development of a modern hatchery program in Alaska was based on a different set of circumstances than that of other hatchery programs. First, while fisheries on wild stocks were at historic low levels in the 1970s, spawning populations were still relatively healthy. Second, spawning and rearing habitats essentially were still in pristine conditions with no dams on major anadromous rivers or lakes and with minimal habitat loss from urbanization or industrial developments. Third, there was a growing awareness of concerns over performance and effectiveness of hatcheries and the potential adverse interactions between cultured and wild salmon stocks in hatchery programs in other areas.

These conditions provided a framework for implementing more conservative approaches to avoid past mistakes in other hatchery programs. Paramount among guidelines in developing policies for Alaska's program was a strong bias favoring wild stocks in situations with potential for adverse hatchery-wild stock interaction. Because natural spawning populations of salmon throughout the state were relatively healthy (although at low levels) with an unspoiled habitat base, the emerging hatchery system at the outset was designed not to supplement or rebuild wild stocks, but to supplement and enhance fisheries. This is a different policy foundation compared with other Pacific Rim salmon hatcheries that were developed as mitigation for losses of salmon production due to dams, overfishing, industrial and urban development, or other forms of habitat deterioration.

Scientists and professionals from ADF&G and other agencies developed special policies for genetics, fish health, pathology, limnology and fish culture procedures for the new hatcheries. Some of these policies were codified into state law. The genetics policy prohibited both interstate transports of live salmonids (including gametes) into Alaska and interregional transport of salmonids within the state (Davis *et al.*, 1985; Davis and Burkett, 1989). Engineering requirements and especially the siting of hatcheries were also carefully considered. Most Alaska hatcheries are located at or near tidewater and are often built on non-anadromous water sources below barrier waterfalls so that freshwater interactions between wild and hatchery salmon are eliminated. Hatcheries in Alaska also made ready use of new fish culture practices and technologies, such as high-density substrate incubation systems, marine net-pens for short-term rearing of pink and chum

salmon fry, floating raceways, and barriered lake systems for natural rearing of anadromous juveniles (Heard, 1998).

Regional Aquaculture Associations

Perhaps the most distinctive feature of modern Alaska hatcheries is that most are managed and operated by private sector associations of fishermen, both commercial and recreational, conservationists, and local civic interests. Developed under state Private Non-Profit (PNP) statutes, these resource stakeholders and associations are allowed to: (1) build and operate hatcheries; (2) assist ADF&G in developing and maintaining regional salmon plans; (3) authorize tax assessments on common property commercially caught salmon (usually 2-3%) to support hatcheries; and (4) provide for the sale of a portion of returning hatchery fish to help cover operational cost and repay state loans (McKean, 1991). This framework has allowed the Alaska salmon hatchery program to evolve into an innovative blend of public and private sector participation.

In the 1970s and early 1980s new hatcheries were operated both by ADF&G and private operators with somewhat duplicate roles (Orth, 1978). During that twenty-year period, however, as the system matured, operations for most public hatcheries were transferred to regional associations. Under these arrangements associations have become responsible for the annual cost of operating the hatcheries while the ADF&G has maintained special programs for genetics, fish health, and limnology for oversight assistance (Koenings, 1993; McNair and Holland, 1994). This close coordination between private sector stakeholders, enhancement, and public resource stewardship, similar in many ways to the prefectural fishermen cooperative system in Japan (Nasaka, 1988), has provided important economic and societal benefits in salmon resource management (Pinkerton, 1994).

The primary purpose of Alaska hatcheries, as required by law, is to benefit common property fisheries. Not all PNP hatcheries, however, are required to be part of regional associations. Statutes allow for individuals or small corporations to build and operate hatcheries at specified sites with production levels that are carefully reviewed and approved through detailed regional planning processes. A paramount issue during planning for all hatchery development has been to establish an enhancement program, based on the available science that protected to the greatest extent possible the abundant and viable wild salmon stocks across the state.

Regional Hatcheries

Currently there are eight regional aquaculture associations in Alaska. Five of these have either built hatcheries or are operating facilities initially built as public hatcheries (Mc Nair and Holland, 1993; Mc Nair, 1999). Three aquaculture associations in southwest and western Alaska do not presently operate hatcheries.

Southeast
Two associations in the southeast region, the Southern Southeast Regional Aquaculture Association (SSRAA), headquartered in Ketchikan, and the Northern Southeast Regional Aquaculture Association (NSRAA), headquartered in Sitka, operate six major production hatcheries in the region. In addition, several federal experimental hatcheries, the Bureau of Indian Affairs (BIA) hatchery on the Annette Island Indian Reservation and ten non-association private hatcheries, produce salmon in this region. Douglas Island Pink and Chum, Inc. (DIPAC) operate the largest non-association hatchery in Juneau. Southeast Alaska hatcheries released over 500 million juvenile salmon in 2000, including the salmon

species (in descending order of magnitude) chum (*Oncorhynchus keta*), pink (*O. gorbuscha*), coho (*O. kistutch*), sockeye (*O. nerka*), and chinook (*O. tshawytscha)*, McNair, 2001).

Prince William Sound
The Prince William Sound Aquaculture Corporation (PWSAC) headquartered in Cordova operates five production hatcheries in the region. The Valdez Fishery Development Association also operates one large non-association hatchery in Valdez. Prince William Sound (PWS) hatcheries released over 700 million juveniles in 2000, including (in descending order of magnitude) pink, chum, sockeye, and coho salmon (McNair, 2001). Hatchery production of sockeye salmon by PWSAC occurs at Main Bay Hatchery within PWS and at Gulkana in the upper reaches of the Copper River System that enters the Gulf of Alaska at the eastern edge of the PWS region. The Gulkana project, one of many unique applications of hatchery technology in Alaska, involves a remote egg incubation system that allows fry to emerge naturally into unutilized sockeye salmon lake nurseries (Roberson and Holder, 1987). Gulkana is also one of the few hatchery operations in Alaska not located at or near tidewater.

Cook Inlet
The Cook Inlet Aquaculture Association (CIAA), headquartered in Soldotna, operates two hatcheries in the Cook Inlet region. The ADF&G operate two hatcheries there and a non-association company operates one. Cook Inlet hatcheries released 85 million juveniles in 2000, including (in descending order of magnitude) pink, sockeye, chinook and coho salmon (McNair, 2001).

Kodiak
The Kodiak Regional Aquaculture Association (KRAA), headquartered in Kodiak, operates the only two hatcheries in this region. In 2000 KRAA released 168 million juveniles, including (in descending order of magnitude) pink, chum, sockeye, and coho salmon (McNair, 2001).

Hatchery Closures
Contrary to the belief that most salmon hatcheries, once built, continue to operate indefinitely, regardless of whether they achieve objectives and reach performance goals (Hilborn, 1992; 1999), a total of 13 hatcheries, 28% of those built since the inception of the Alaska program, have been closed (Fig. 2).

Closures have occurred in all regions due to a variety of factors including: disease or genetic concerns for protecting wild stocks; avoiding major disease consequences in hatcheries; other biological concerns in the hatchery; management concerns over mixed stock fisheries; and cost efficiencies or other economic issues.

Statewide Commercial Harvest

A look at the relative contributions of each species of wild and hatchery salmon to Alaska commercial catches provides a better understanding of how enhancement operations complement common property harvest in statewide fisheries.

Figure 2. Location and names of thirteen Alaska hatcheries that have been closed since the beginning of the current salmon enhancement program.

There are many hatcheries that do not contribute to fisheries. Many hatcheries emphasize some species over others. The focus on one species over another is based on both historical precedence of salmon fisheries within regions and on comprehensive regional planning.

Pink Salmon
Pink salmon are the most abundant species of pacific salmon in Alaska, accounting for 40-70% of the total commercial harvest each year. Between 1970 and 2000 pink salmon comprised 57% of the average annual commercial harvest. Pink salmon are mostly harvested by purse seines in Southeast, South-central (including PWS) and Kodiak Island regions of the state. Over the past six-year period (1995-2000) commercial catches of wild pink salmon have ranged from 25 to over 100 million fish, while catches of hatchery-reared fish ranged from 23-47 million (Fig. 3). In 2000 wild and hatchery pink salmon contributions to the fishery were about equal. Hatchery production of pink salmon is greatest in the PWS Region where 60-80% of the commercial harvest in recent years consisted of hatchery-reared fish, an issue of some controversy.

Chum Salmon
Chum salmon are harvested commercially by purse seines, drift and set gillnets, and, in large Western Alaska rivers, by fishwheels. Between 1970 and 2000 chum salmon have accounted for 10% of Alaska's total salmon harvest. Between 1995 and 2000 the average annual chum salmon harvest across Alaska totaled 20 million fish, with the commercial catch in 2000 above this average at a record 24 million (ADF&G, 2001).
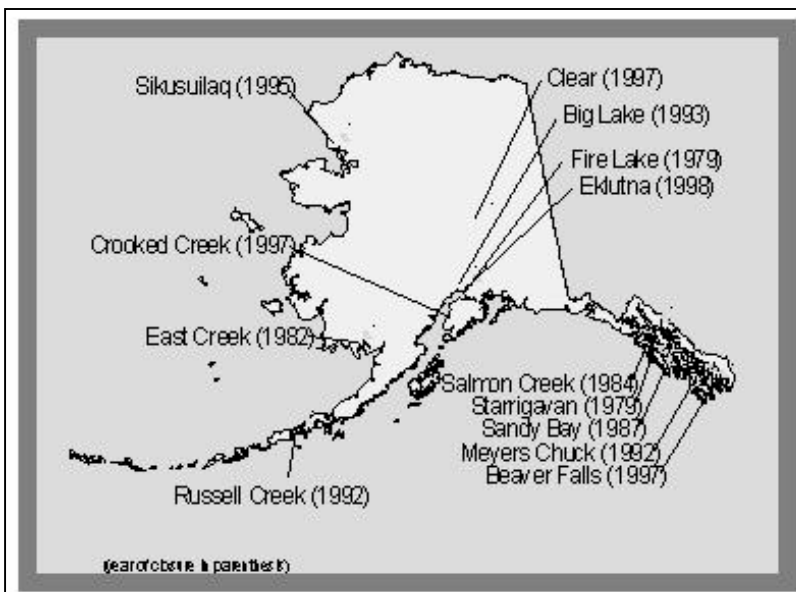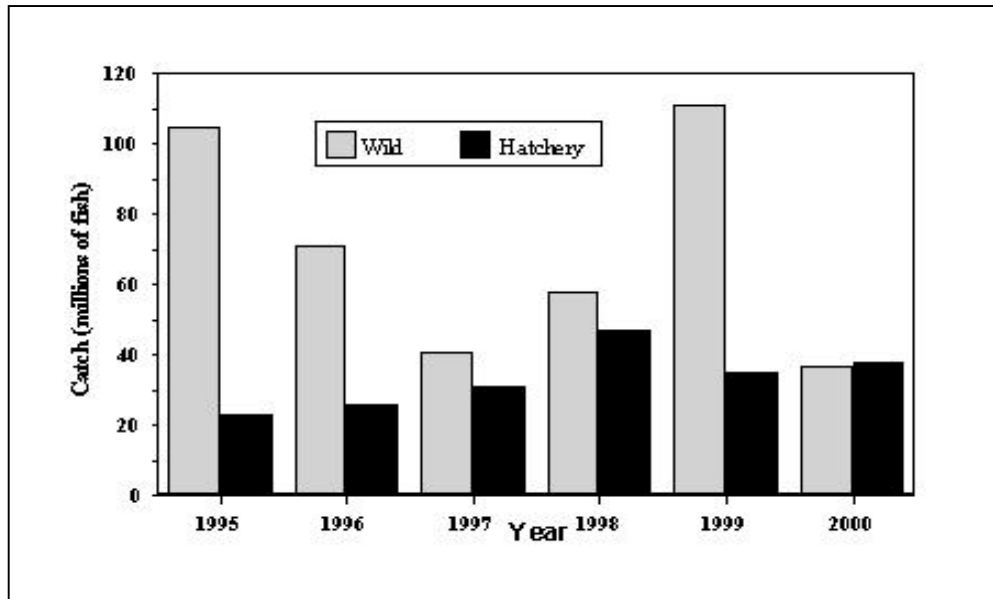
Figure 3. Statewide contributions of wild and hatchery pink salmon in Alaska commercial fisheries 1995-2000.

In contrast to pink salmon, statewide hatchery production of chum salmon in Alaska exceeds wild stock contributions in commercial catches. Chum salmon hatcheries during the past six years have produced from 9-18 million fish, while wild stocks have contributed 4-9 million fish annually to the commercial harvest (Fig. 4). Currently 60-70% of the commercial harvest of chum salmon in the state occurs in the Southeast Region where hatcheries produced an even greater portion (80%) of the catch in 2000 (McNair, 2001).
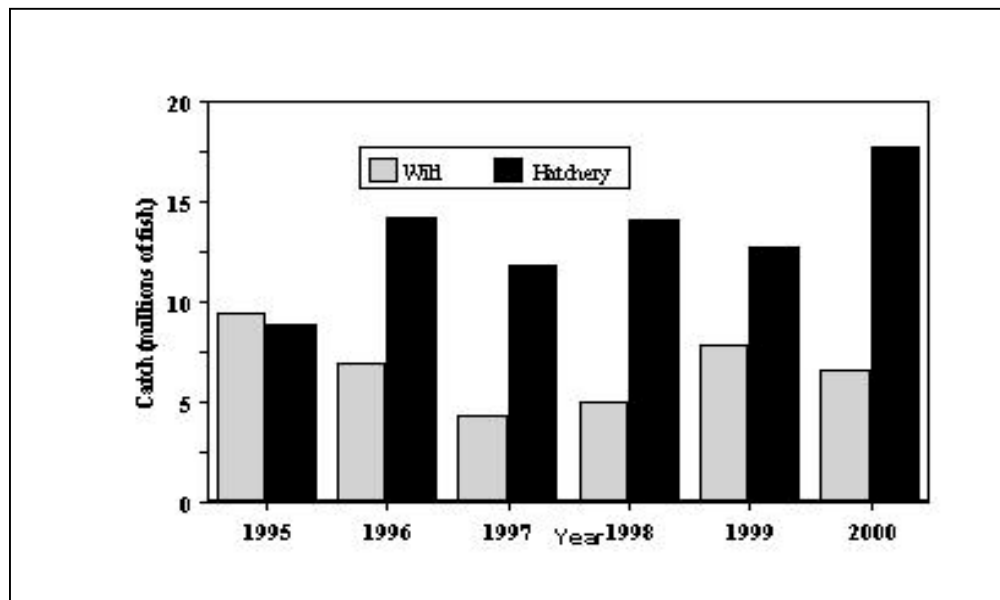

Figure 4. Statewide contributions of wild and hatchery chum salmon in Alaska commercial fisheries between 1995-2000.

Coho Salmon
Coho salmon in Alaska are caught commercially by purse seines in the Southeast and South-central regions, by hand and power troll gear in the Southeast region, and by drift or set gillnets in all regions. During the period 1995-2000 commercial catches of coho salmon statewide ranged from 3-6 million fish, 20-25% of those being of hatchery origin (Fig. 5). Commercial harvest in 2000 totaled 4.2 million fish, somewhat less than recent average harvest levels, but well above the record low catches in the 1970s (ADF&G, 2001).
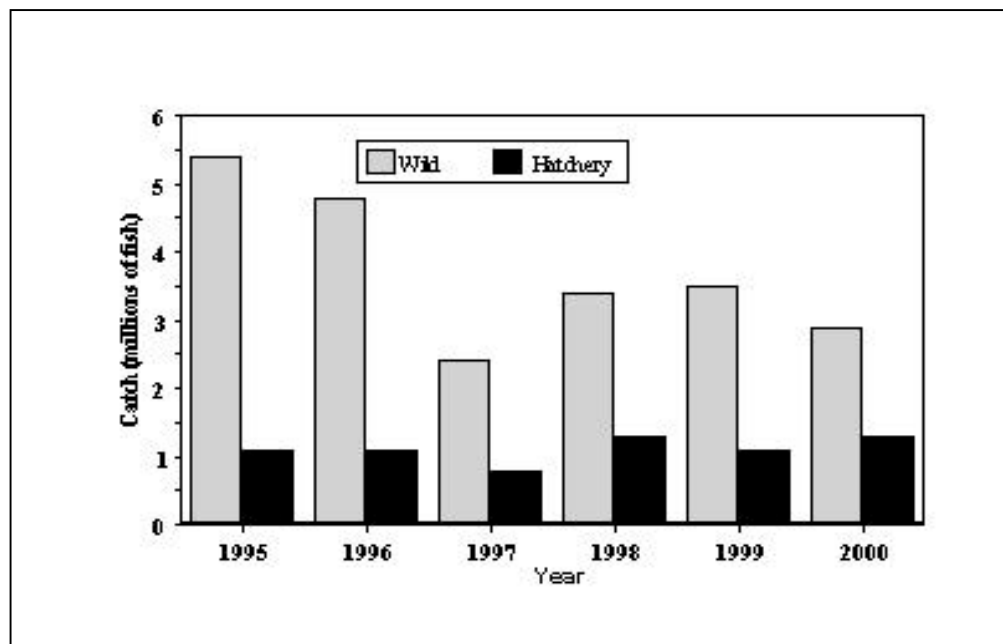

Figure 5. Statewide contributions of wild and hatchery coho salmon in Alaska commercial fisheries between 1995-2000.

About 60 percent of the statewide harvest of coho salmon come from the Southeast region. This relatively high commercial harvest was due to generally favorable ocean survival conditions and good returns in the Southeast region where 3.0 million or more coho salmon from hatchery and wild stock production were caught in three out of the last five years. Hatcheries in Southeast produce around 30% of the annual commercial catch.

Sockeye Salmon
Sockeye salmon, harvested commercially by purse seine in Southeast, Kodiak and Chignik fisheries and by drift gillnet or set gillnet throughout the state, are the second most abundant species caught in Alaska fisheries and account for about 28% of the total salmon harvest in recent years. The largest fisheries for sockeye salmon occur in Bristol Bay, Cook Inlet, Alaska Peninsula-Aleutian Islands, and Kodiak regions while other regions, also have fisheries for this species.
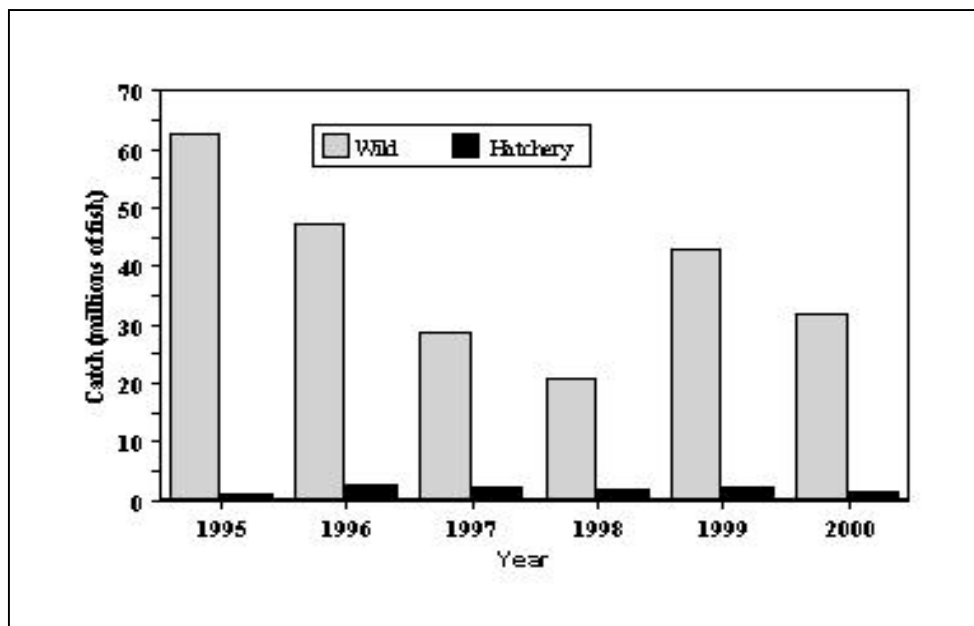
Figure 6. Statewide contributions of wild and hatchery sockeye salmon in Alaska commercial fisheries between 1995-2000.

Statewide one to two million sockeye salmon from hatcheries are caught by commercial fisheries, whereas wild stocks have produced from 23 to 62 million fish annually over the past six-year period (Fig. 6). Sockeye salmon provide greater dollar value to Alaska commercial fishermen than all other salmon species combined, usually yielding from 60-70% of the ex-vessel value of the annual commercial harvest. The Bristol Bay sockeye salmon fishery in Southwest Alaska is the most valuable capture fishery for salmon in the world, yielding $300-400 million (ex-vessel) per year in the 1980s and early 1990s. In more recent years, however, world salmon prices have declined significantly and ex-vessel values of Bristol Bay sockeye salmon averaged only $88 million during the three-year period between 1998-2000 (ADF&G, 2001).

Chinook Salmon
The annual commercial harvest of chinook salmon in Alaska has averaged between 400 and 700 thousand fish in recent years with hatcheries producing from 75,000-100,000 of the total (Fig. 7). Chinook salmon, like coho salmon, are commercially harvested by purse seines in the Southeast and Southcentral regions, by drift or set gillnets in all regions, and by hand and power troll gear in the Southeast region. In addition, fishwheels harvest chinook salmon in Western Alaska rivers for commercial sales and subsistence uses.
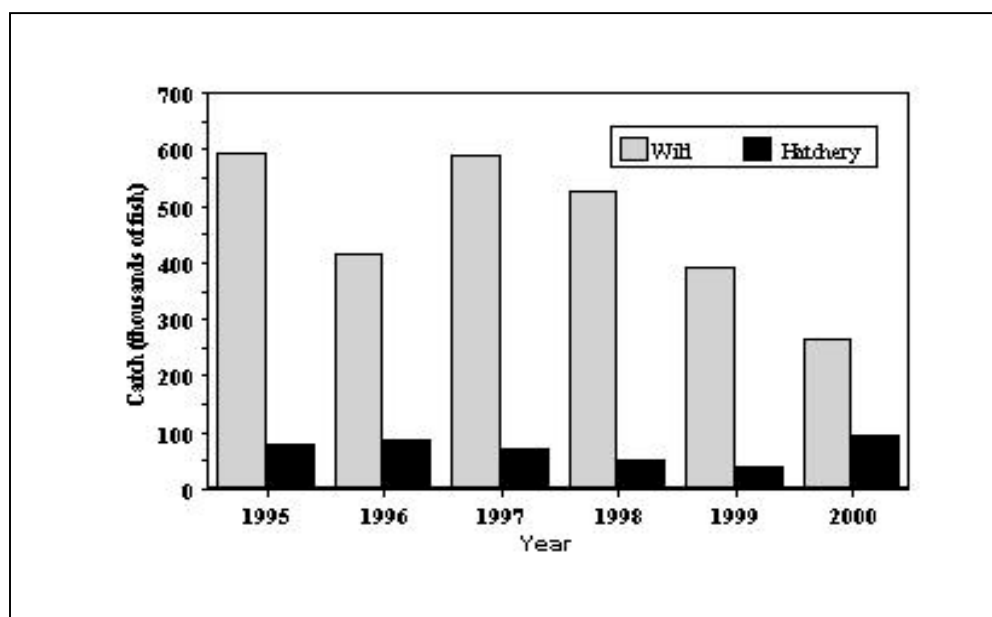
Figure 7. Statewide contributions of wild and hatcherty Chinook salmon in Alaska commercial fisheries between 1995-2000.

Generally, chinook salmon are the first species each year to begin spawning migrations into Alaska rivers. Only in a few Bristol Bay and Western Alaska rivers are fisheries permitted to directly target these early returning runs. In fisheries targeting on other salmon, however, chinook salmon are often taken incidentally. Sockeye salmon migrations into many larger river systems begin during the later portion of chinook salmon runs into the same rivers. In these cases, for example in certain Cook Inlet, Southeast rivers, and in the Copper River near Cordova, fisheries that target on sockeye salmon may catch significant numbers of chinook salmon. Some of these fisheries may have quotas limiting the chinook salmon catch. The region-wide chinook salmon harvest in the Southeast, where significant numbers of non-Alaska origin fish are also caught, is normally regulated by an abundance-based management system under provisions of the Pacific Salmon Treaty. Because of Treaty related limits on commercial and recreational catches much of the hatchery focus on chinook salmon in Alaska is in the Southeast region (Heard *et al.,* 1995).

Recreational Fisheries

While all species of salmon are important in Alaska sport fishing circles, coho, sockeye, and chinook salmon are the most popular target species in recreational fisheries throughout the state. Coho salmon were the most popular sport caught salmon in 1999, representing 44% of the 1.4 million salmon caught by recreational fishermen, followed by sockeye salmon (26%), chinook salmon (13%), pink salmon (12%), non-anadromous landlocked salmon (2%) and chum salmon (2%, Howe *et al.,* 2001). Roughly one third of the total Alaska sport catch of coho salmon in 1999 originated from hatcheries (McNair, 2000). Alaska hatcheries are providing important recreational fishing opportunities in many of the urban areas of the state, including Anchorage, Juneau, Ketchikan, Petersburg, Sitka, and Valdez.

Discussion

During the six-year period between 1995-2000 the additional contribution from Alaska's statewide hatchery production to commercial harvests over wild stocks was greatest for chum salmon followed by pink, coho, chinook and sockeye (Fig. 8). Over the past two decades wild stocks have made dramatic recoveries from their record lows, and together with implementation of the current hatchery program, commercial harvests have reached record highs. Hatcheries are now making significant contributions to commercial and recreational fisheries in several regions of the state, most notably in the Southeast and PWS regions.
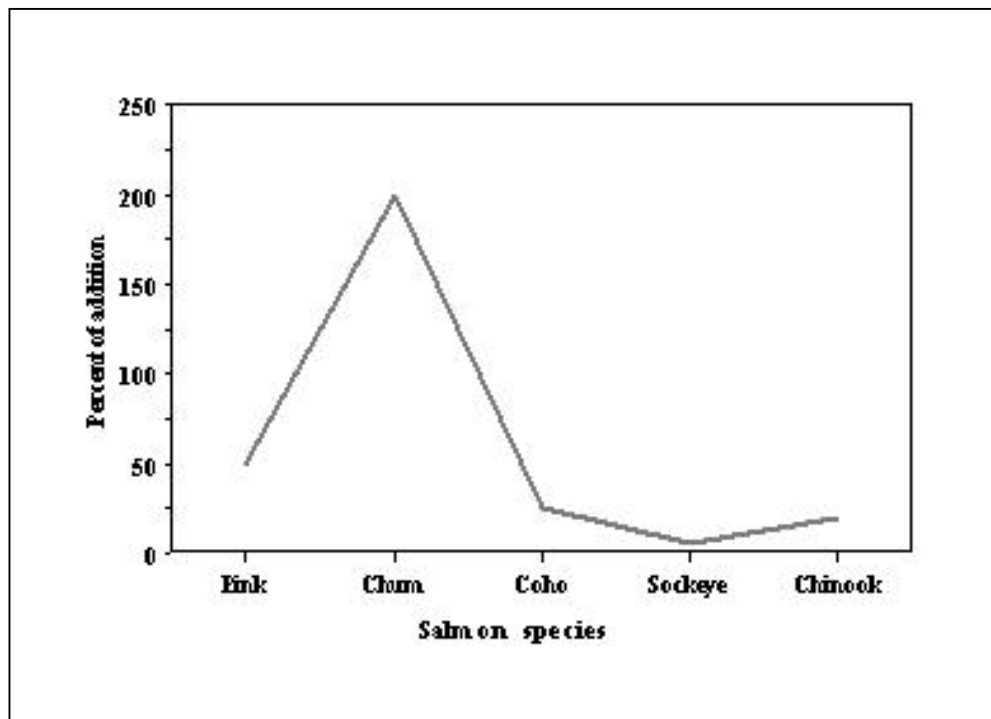

Figure 8. Additional statewide hatchery contributions of salmon by species over wild stock harvest in Alaska commercial fisheries between 1995-2000.

While depressed fisheries dependent on wild stocks were the impetus for the current hatchery program, a novel system of public and private non-profit hatcheries has evolved to augment, not replace, wild salmon in common property fisheries. One goal of this program is to smooth out sharp downside fluctuations in long-term abundance cycles that are strongly correlated with climatic-driven changes in the marine environment (Beamish and Bouillion, 1993; Francis and Hare, 1994; Hare and Francis, 1995; Mantua *et al.*, 1997; Beamish *et al.*, 1998). Although hatcheries in Alaska have enjoyed considerable success during their first twenty-year history, because of these long-term cycles, a longer time horizon is likely needed, as suggested by Hilborn and Winton (1993) to properly evaluate this kind of enhancement program.

Changes in marine environments that influence ocean survival of young salmon can also affect freshwater life stages due to colder or drier winters reducing the survival of eggs and fry. Under these conditions hatchery salmon, while subjected to the same marine conditions as wild salmon, will have freshwater survival advantages due to controlled conditions during these life

stages. As noted by Smoker and Linley (1997) the "...hatchery program was not developed to ameliorate poor marine survival. Rather, it was developed to ameliorate limitations of the freshwater environment...".

Some scientists now believe major environmental changes are underway and that the twenty-year high survival rate of Alaska salmon may be declining, due to climatic pattern shifts in the North Pacific Ocean (Klyashtorin, 1998; Klyashtorin and Rukholv, 1998; Noakes *et al.*, 1998; Welsh *et al.*, 1998). Evidence also suggests there may be a climate-driven inverse pattern of salmon production between Alaska and the West Coast (Hare *et al.*, 1999). Recent increases in numbers of West Coast salmon, therefore, could indicate a declining trend for Alaska salmon. However, the jury is still out on this issue for although Alaska's commercial catches did decline for three years following the record 1995 harvest, landings in 1999 reached 217 million fish, essentially matching the peak harvest year. Landings fell to 137 million salmon in 2000 (Fig. 1), but preliminary data for 2001 indicate a rebound to173 million fish (ADF&G, 2001). Although salmon resources in Alaska generally are still healthy, in some areas, notably Western Alaska including Bristol Bay, the Kuskokwim and Yukon River drainage, many stocks are badly depressed resulting in major economic hardships in those regions. Interestingly, these are areas with little or no enhancement effort.

Successes of Alaska's hatcheries has involved some controversy and the program has been critically challenged in two areas, the large pink salmon hatchery program in PWS and the chum salmon hatchery program in the Southeast region. Eggers *et al.* (1991) reviewed trends of wild-hatchery pink salmon interactions in PWS and raised concerns over declines in wild stock escapements and the ability to conduct fisheries on large hatchery runs concomitant with reaching target escapement goals for wild stocks. Hilborn (1992) also criticized pink salmon hatchery production in PWS suggesting the program was without merit and "...should be terminated". Kron (1995), however, revisited the issue of declining wild stock escapements in PWS and indicated that new analyses of available data suggested escapements were higher than previously thought and that they were consistent with long term, pre-hatchery levels. He further noted that large scale marking programs of PWS hatchery pink salmon were enabling managers to conduct fisheries while achieving escapement goals. A careful, contemporary and historic analysis of PWS fisheries and escapement patterns (Smoker and Linley, 1997) suggested there was no credible evidence that hatcheries have harmed wild stocks.

Hilborn (1999) asserted that PWS hatcheries have not increased pink salmon production above the current high levels in the Southeast region where there is minimal hatchery production of this species. In a more rigorous analysis Hilborn and Eggers (2000) argue that pink salmon hatchery program in PWS has essentially replaced wild stock production that would have occurred in the absence of hatcheries. Wertheimer *et al.* (2001), however, analyzed the same data sets along with other factors and concluded that PWS hatcheries were supplementing wild stock production with a net gain of 17.5-23.7 million pink salmon annually to fisheries in that region.

Some components of the Hilborn and Eggers (2000) argument included temporal comparisons of hatchery and wild pink salmon production in PWS with other regions. In PWS, as hatchery production was getting started, wild stock productivity was near record high levels. As hatchery production began increasing, wild stocks in PWS after a seven-year period of unusually high production (1979-1985), began declining while wild stock production in other regions continued at relatively high levels. This declining trend in PWS wild stocks persisted as hatchery production reached current high levels. Superficially this pattern gives the appearance of a possible cause and effect relationship between hatchery production and decline in wild stocks (Fig. 9).
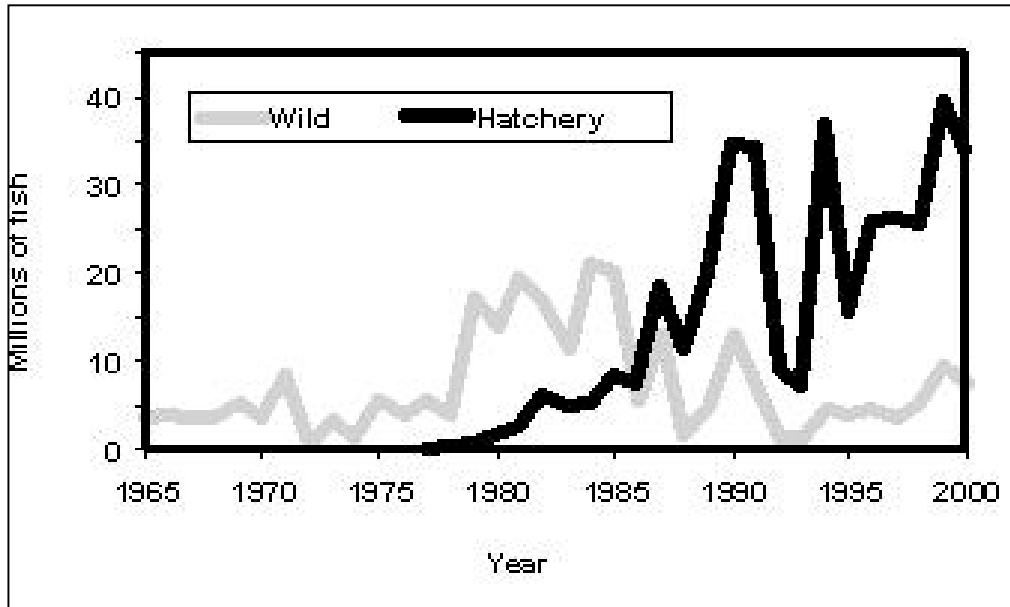
Figure 9. Commercial harvest of wild and hatchery pink salmon in Prince William Sound, Alaska between 1965-2000 (Wertheimer *et al.,* in press).

Another element of concern, as hatchery releases of juveniles in PWS increased, was whether decreases in productivity among wild stocks, expressed as adult returns per spawner, was the result of density-dependent responses to the large releases of hatchery fry. Hatchery survivals have been lower in 1986-1995, when hatchery fry releases were higher, relative to survivals in an earlier period (1977-1985), when hatchery releases of fry were smaller (Fig. 10).
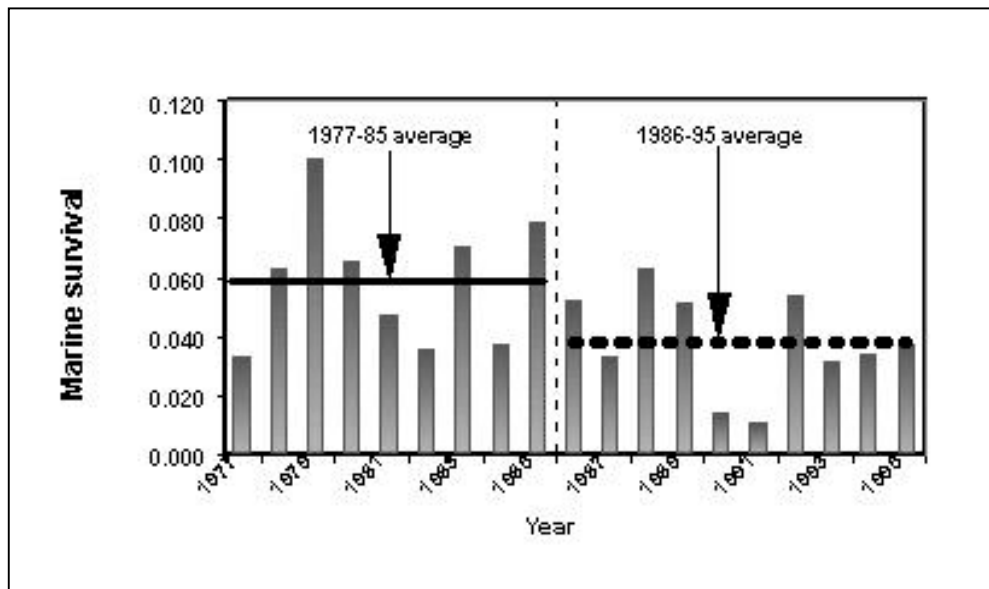
Figure 10. Marine survivals of pink salmon hatchery releases of fry in Prince William Sound, 1977-1995, showing two periods with different average values (Wertheimer *et al.,* in press).

Correlation analyses of hatchery marine survivals to hatchery fry releases, however, strongly suggested a density-independent response to marine conditions (Fig. 11) indicating that other factors caused the decline in wild stock productivity in PWS (Wertheimer *et al.,* in press).

Figure 11. Correlation of Prince William Sound hatchery pink salmon marine survivals from hatchery fry releases during the 1975-1998 brood years (Wertheimer *et al.,* in press).

In further analyses Wertheimer *et al.*, (in press) examined a suite of eleven concurrent biophysical variables related to PWS pink salmon production, including releases of hatchery fry and numbers of wild spawners, in a stepwise regression model. Data for some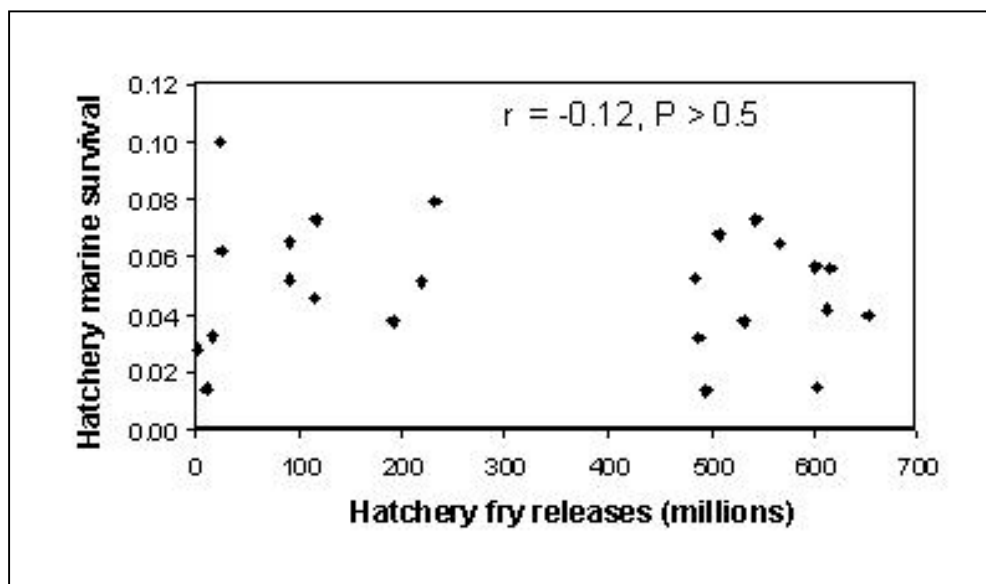 variables was only available for restricted time intervals. These analyses found that hatchery survival, as an index of environmental conditions affecting wild stock survival, was the most significant factor that alone accounted for 49-60% of variability in wild stock productivity. Variables representing other marine environmental conditions, PWS zooplankton index (representing availability of pink salmon prey) and Gulf of Alaska sea surface temperature (SST) were also important functions in explaining the variation in wild stock productivity. Based on these analyses, Wertheimer *et al.* (in press) concluded that pink salmon hatcheries in PWS provide a net gain of up to 25.3 million fish per year. They also noted that under certain worst case conditions there might be up to a 4.5 million annual wild stock yield loss due to hatchery releases. However, even under these conditions there still was a 20.8 million net gain in available pink salmon for fishery harvest in the region. Therefore, while some other regions in Alaska continued to have high wild stock productivity after the 1986 decline in wild stocks began in PWS (Fig. 9), biophysical variables affecting PWS are the major causes of wild stock declines rather than displacement from hatchery production. This conclusion is also consistent with a study by Pyper *et al.* (2001) that found that environmental processes affecting temporal variation in survival rates of pink salmon operate at regional spatial scales rather than larger ocean-basin scales.

Controversy over Southeast Alaska chum salmon hatchery production involves speculation that hatchery fish from this region during their marine life history somehow have a competitive and deleterious impact on wild chum salmon productivity in Kuskokwim and Yukon River systems. Some fishermen and civic leaders from Western Alaska have argued that a reduction in chum salmon hatchery production in the Southeast Region would help Western Alaska stocks recover. This rationale is based on the overlap in oceanic distribution of stocks from these two regions during part of their marine life cycles. Chum salmon stocks from Western Alaska rivers currently are at very depressed low levels, however, causes for this decline are largely unknown. Preliminary indications suggest ecological changes in the Bering Sea ecosystem may have significant negative impacts on juvenile salmon from Western Alaska rivers during early sea life. New freshwater and marine research programs are being implemented to better understand causes of chum salmon declines in this region. However, presently there is no credible evidence that Southeast hatchery chum salmon have a deleterious impact on Western Alaska stocks.

Statewide, the major problem faced by Alaska's salmon industry is a continuing decade-long decline in commercial value of salmon caught in fisheries. Over a recent thirteen-year period, 1988-2000, commercial catches of Alaska salmon have averaged around 145 million fish with 15-40% of this harvest derived from hatcheries. The ex-vessel values paid to fishermen during this period, however, have steadily declined from over 500 million dollars annually to 250 million dollars (Fig. 12).
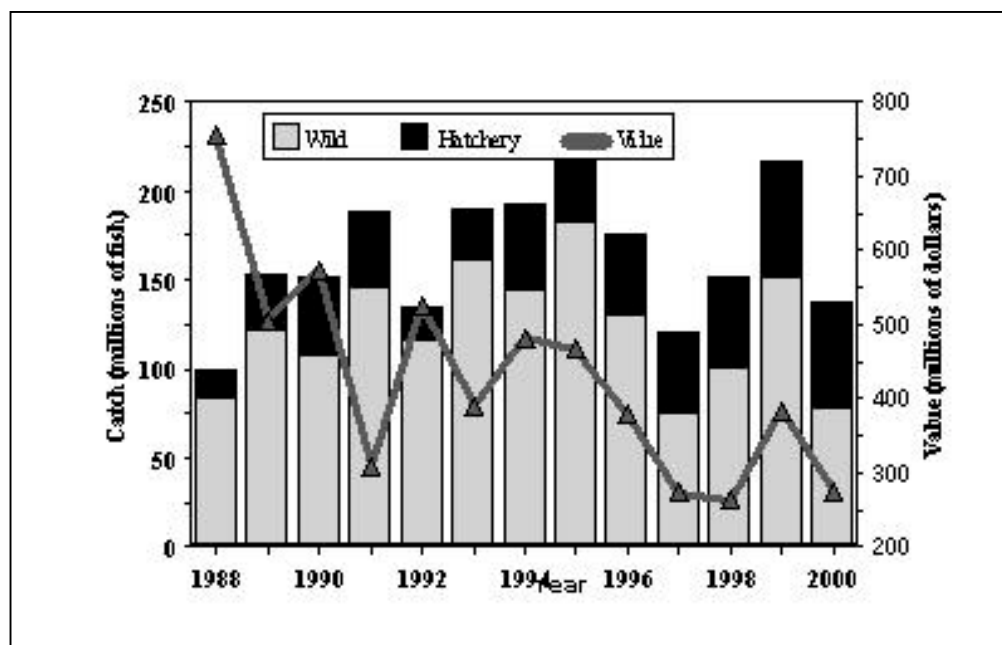
Figure 12. Statewide contributions of wild and hatchery salmon in Alaska commercial fisheries and ex-vessel values paid to fishermen, 1988-2000.

Many factors interact to determine prices paid to salmon fishermen in capture fisheries. Most important has been the overall supply and demand economics driven by continued increases in world supplies of farmed salmon produced by many countries (Knapp, 1998). While worldwide capture fisheries for salmon since 1985 have remained relatively stable at around 600,000-900,000 t annually, farmed salmon production has grown steadily during the same period from around 50,000 t to over one million t in 2001 (Fig. 13). For the first time, farmed production exceeded capture fisheries in 1998, and there is no indication of any change in this increasing availability of farmed salmon (Knapp, 2001). The negative impacts of this trend on the value of commercial salmon fisheries in Alaska and in Japan (Kaeriyama and Urawa, 1993) have created serious economic hardships in capture fisheries for salmon throughout the North Pacific Rim.

As recently as 1982 Alaska salmon held the dominate share (45%) of the total world supply of salmon, but fifteen years later, in spite of a period of record high commercial catches, Alaska's share had dropped to 19% of world supplies (Spiess, 1998). There are more salmon available today in commercial markets than ever before as world salmon production approaches two million tons. However, in spite of a generally negative economic outlook for commercial salmon fisherman based on capture fisheries, the Alaska enhancement program, focused on regional aquaculture associations and private sector involvement is currently providing significant, positive impacts to regional economies (McDowell, 2001; DIPAC, 2001).
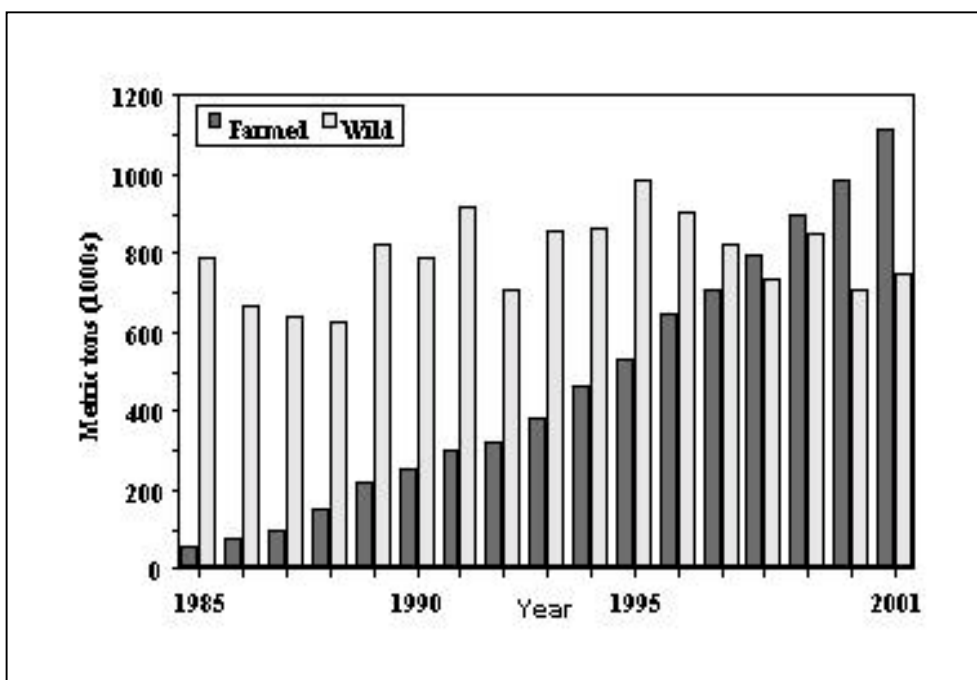
Figure 13. Worldwide production of farmed salmon and wild salmon caught in capture fisheries, 1985-2001. Wild salmon in this context includes fish from enhancement and hatchery programs. Data from 2001 are provisional.

Conclusions

Alaska salmon runs and the fisheries that depend on them are unique in that nowhere else around the Pacific Rim does such a relatively large and successful hatchery program coexist with abundant and healthy wild stocks of salmon. Salmon populations in Alaska, in general, are strong, healthy, and currently are at or near record high levels (Burger and Wertheimer, 1995; Baker *et al.*,1996; Wertheimer, 1997; ADF&G, 2001), in spite of sharp fluctuations in some stocks in some regions in some years (Kruse, 1998). After two decades of operations in several regions Alaska hatcheries are successfully making meaningful contributions to fisheries with little, if any, evidence of significant, detrimental impacts either on the environment or on wild stocks.

Salmon management in Alaska is strongly directed by law, policy, and regulation to maintain adequate wild stock escapements and preserve existing pristine habitats. Even if no hatchery fish are involved, management policies are directed at reaching target escapement goals for wild runs rather than any predetermined harvest goal for the benefit of fisheries. Escapement-based management and strong habitat laws preventing catastrophic habitat losses, together with a carefully implemented and conservative hatchery program, are key hallmarks of Alaska's commitment to maintaining healthy salmon runs and fisheries that depend on these resources (Holmes and Burkett, 1996).

The current successful hatchery program is playing a significant role in helping meet this commitment. Only time will tell if the present favorable balance between wild and hatchery salmon production will continue into the future. Alaska's modern hatchery program started with the onset of higher salmon survivals due to favorable conditions in the marine environment. It is essential to continue this program along with ongoing research and careful evaluation to correctly assess its

effectiveness when long-term cyclic and environmental influences change to less favorable conditions.

Literature Cited

ADF&G (Alaska Department of Fish and Game). 2001. Catch, effort, and value statewide harvest; recent years harvest statistics. Blue Sheets. http://www.cf.adfg.state.ak.us/

Baker, T. T., A. C. Wertheimer, R. D. Burkett, R. Dunlap, D. M. Eggers, E. I. Fritts, A. J. Gharrett, R. A. Holmes, and R. L. Wimot. 1996. Status of Pacific salmon and steelhead escapements in southeastern Alaska. *Fisheries* 21(10):6-19.

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

Beamish, R., D. Noakes, G. McFarlane, and J. King. 1998. The regime concept and recent changes in pacific salmon abundance. p.1-3. <u>In</u>: K. Myers (ed.) Workshop on Climate Change and Salmon Production. N. Pac. Anadr. Fish Comm. Tech. Rept.Vancouver, Can.

Burger, C. V., and A. C. Wertheimer. 1995. Pacific salmon in Alaska. p. 343-247. <u>In</u>: E.T. La Roe, G.S. Farris, C. E. Puckett, P. D. Doran, and   J. J. Mac (eds.). Our living resources: a report to the nation on the distribution, abundance, and health of U. S. plants, animals, and ecosystems. U.S. Dept. Interior, Natl. Biol. Surv. Washington, D.C. 530p.

Byerly, M., B. Brooks, B. Simonson, H. Savikko, and H. J. Geiger. 1999. Alaska commercial salmon catches, 1878-1997. Reg. Inform. Rept. 5J99-05. 66p. Alaska Dept. Fish and Game Div. Comm. Fish. Box 25526, Juneau, AK 99802

Davis, B., and seven co-authors. 1985. Alaska Dept. of Fish and Game Genetic Policy. Genetic Policy Review Team. ADF&G, Juneau, AK. 99802

Davis, B. and R. Burkett. 1989. Background of the genetic policy of Alaska Department of Fish and Game. Alaska Department of Fish and Game. FRED Report 95. Juneau, AK. 99802.

DIPAC (Douglas Island Pink and Chum, Inc.). 2001. Calculating the DIPAC difference in Southeast Alaska. DIPAC Network News. 2697 Channel Dr., Juneau, AK. 99801 8 p.

Eggers, D. M., L. R. Peltz, B. G. Bue, and T. M. Willette. 1991. Trends in abundance of hatchery and wild stocks of pink salmon in Kodiak Island, Cook Inlet, and Prince William Sound, Alaska. Professional Paper 35. Alaska Dept. Fish and Game, Division of Commercial Fisheries, Juneau, AK. 99802

Francis, R. C., and S. R. Hare. 1994. Decadal-scale regime shifts in the large marine ecosystems of the Northeast Pacific: a case for historical science. *Fish. Oceanogr.* 3: 279-291.

Hare, S. R., and R. C. Francis. 1995. Climate change and salmon production in the Northeast Pacific Ocean. p.357-372. <u>In</u>: R. J. Beamish (ed.) Climate change and northern fish populations. *Can. Spec. Pub. Fish. Aquat. Sci.* 121.

Hare, S. R., N. J. Mantua, and R. C. Francis. 1999. Inverse production regimes: Alaska and West Coast Pacific salmon. *Fisheries* 24:6-14.

Hare, S. R. and N. Mantua. 2000. Empirical evidence for North Pacific [climatic] regime shifts in 1977 and 1989. *Prog. Ocanogr*. 47:103-145.

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

Heard, W. R. 1998. Unusual culture practices for Pacific salmon in Alaska. p. 102-112. In*:* G. A. Wedemeyer (ed.) Second U.S.-U.S.S.R. Symposium, Reproduction, Rearing, and Management of Anadromous Fishes. U.S. Dept. Interior, Biological Resources Div., U.S. Geological Survey. Seattle, Wash.

Hilborn, R. 1992. Hatcheries and the future of salmon in the northwest. *Fisheries* (Bethesda) 17:(1) 5-8.

Hilborn, R. and J. Winton. 1993. Learning to enhance salmon production: lessons from the salmonid enhancement program. *Can. J. Fish. Aquat. Sci*. 50:2043-2056.

Hilborn, R. 1999. Confessions of a reformed hatchery basher. *Fisheries* 24 (5):30-31.

Hilborn, R. and D. Eggers. 2000. A review of the hatchery programs for pink salmon in Prince William Sound and Kodiak Island, Alaska. *Trans. Am. Fish. Soc*. 129:333-350.

Howe, A. L., R. J. Walker, C. Olnes, K. Sundet, and A. E. Bingham. 2001. Revised Edition: Participation, catch, and harvest in Alaska sport fisheries during 1998. ADF&G, Fishery Data Series No. 99-41 (revised) Anchorage, AK.

Holmes, R. A. and R. D. Burkett. 1996. Salmon stewardship: Alaska's perspective. *Fisheries* 21:36-38.

Kaeriyama, M. and S. Urawa. 1993. Future research by the Hokkaido Salmon Hatchery for the proper maintenance of Japanese salmonid stocks, p. 57-62. In*:* Y. Ishida, Y. K. Nagasawa, D. Welch, K. W. Myers, and A. Shershnev (eds.). Proceedings of the International Workshop on Future Salmon Research in the North Pacific Ocean. Special Pub. Nat .Res. Inst. Far. Seas Fisheries. 20. Shimizu, Japan.

Klyashtorin, L. B. 1998. Cyclic climatic changes and Pacific salmon stock fluctuations: a possibility for long-term forecasting. p. 6-7. In: K. Myers (ed.) Workshop on Climate Change and Salmon Production. N. Pac. Anadr. Fish Comm. Tech. Rept. Vancouver, Can.

Klyashtorin, L. B. and F. N. Rukhlov. 1998. Long-term climate change and pink salmon stock fluctuation. *N. Pac. Anadr. Fish Comm. Bull*. No. 1:464- 479.

Knapp, G. 1998. The future of wild salmon. Spec. Resource Supp., Alaska Econ. Rept. and Legislative Digest. No. 19.

Knapp, G. 2001. Estimates of world salmon supply. Univ. of Alaska, Anchorage, AK. Excel datafiles.

Koenings, J. 1993. Editorial. Alaska's wildlife. Alaska Dept. Fish Game. 25: (2)1. Juneau, AK. 99802

Kron, T. 1995. Prince William Sound salmon enhancement programs and considerations relative to wild stocks. p.49-52. In: M. R. Collie and J. P. McVey (eds.) Interactions Between Cultured Species and Naturally Occurring Species in the Environment. U S- Japan Coop. Prog. Nat. Res. Tech. Rep. 22. Alaska Sea Grant Rep. 95-03.

Kruse, G. H. 1998. Salmon run failures in 1997-1998: a link to anomalous ocean conditions? *Alaska Fish. Res. Bull*. 5:55-63.

Mantua, N. J., S. R. Hare, Y. Zhang, J. M. Wallace, and R. C. Francis. 1997. A Pacific inter-decadal climate oscillation with impacts on salmon production. *Bull. Am. Meteorological Soc.* 78:(6)1069-1079.

McDowell Group Inc. 2001. Economic impacts of the Northern Southeast Regional Aquaculture Association. Juneau, AK. 99801. 25p.

McKean, M. 1991. FRED 1990 annual report to the Alaska state legislature. ADF&G FRED Rep. 109. 167 p.

McNair, M. and J. S. Holland. 1993. FRED 1992 annual report to the Alaska state legislature. ADF&G FRED Rep. 127. 102 p.

McNair, M. and J. S. Holland. 1994. Alaska fisheries enhancement programs 1993 annual report. Alaska Dept. Fish and Game Comm. Fish. Management and Dev. Div. Juneau, AK. 99802. 43 p.

McNair, M. 1999. Alaska salmon enhancement program 1998 annual report. Alaska Dept. Fish and Game Div. Comm. Fish. Reg. Info. Rept. 5J99-02. Juneau, AK. 99802. 35p.

2000. Alaska salmon enhancement program 1999 annual report. Alaska Dept. Fish and Game Div. Comm. Fish. Reg. Info. Rept.5J00-02, Juneau, AK. 99802. 34 p.

2001. Alaska salmon enhancement program 2000 annual report. Alaska Dept. Fish and Game, Regional Inf. Rept. 5J01-01. Juneau, AK. 99802. 35 p.

Nasaka, Y. 1988. Salmonid programs and public policy in Japan. p. 25-31. In: W. J. McNeil (ed.). Salmon production, management and allocation- biological, economic, and policy Issues. Oregon State Univ. Press, Corvallis, OR.

Noakes, D. J., R. J. Beamish, L. Klyashtorin, and G. A. McFarlane. 1998. On the coherence of salmon abundance trends and environmental factors. *N. Pac. Anadr. Fish Comm. Bull*. No.1: 454-463.

Orth, F. L. 1978. The Alaska salmon enhancement program: imperatives for economic success. Univ. Alaska Sea Grant Rep. 78-1: 13 p.

Pinkerton, E. 1994. Economic and management benefits from the coordination of capture and culture fisheries: the case of Prince William Sound pink salmon. *N. Am. J. Fish. Mgt*. 14:262-277.

Pyper, B. J., F. J. Muster, R. M. Peterman, D. J. Blackbourn, and C. C. Wood. 2001. Spatial covariation in survival rates of Northeast Pacific pink salmon (*Oncorhynchus gorbuscha*). *Can. J. Fish. Aquat. Sci*. 58:1501-1515.

Roberson, K. and R. R. Holder. 1987. Development and evaluation of a streamside sockeye salmon (*Oncorhynchus nerka*) incubation facility, Gulkana River, Alaska, p. 191-197. In: H. D. Smith, L. Margolis, and C. C. Wood (eds.). Sockeye salmon(*Oncorhynchus nerka*) population biology and future management. *Can. Spec. Publ. Fish. Aquat. Sci*. 96.

Smoker, W. W. and T. J. Linley. 1997. Are Prince William Sound salmon hatcheries a fool's bargain? *Alaska Fish. Res. Bull.* 4:5-78.

Spiess, B. 1998. Alaska braces to compete in salmon markets. *Pacific Fishing*. July 1998: pp.24-25.

Welch, D. W., B. R. Ward, B. D. Smith, and J. P. Eveson. 1998. Influence of the 1990 ocean climatic shift on British Columbia steelhead (*O. mykiss*) and coho (*O. kisutch*) populations. p. 8-10. <u>In</u>*:* K. Myers (ed.) Workshop on Climate Change and Salmon Production. N. Pac. Anadr. Fish Comm. Tech. Rept. Vancouver, Can.

Wertheimer, A. C. 1997. Status of Alaska salmon, p. 179-197. <u>In</u>: D. J. Stouder, P. A. Bisson. and R. J. Naiman (eds.) Pacific salmon and their ecosystems, status and future options. Chapman and Hall, New York.

Wertheimer, A. C., W. W. Smoker, T. L. Joyce, and W. R. Heard. 2001. Comment: A review of the hatchery program for pink salmon in Prince William Sound and Kodiak Island, Alaska. *Trans. Am. Fish. Soc*. 130:712-720.

Wertheimer, A. C., W. R. Heard, and W.W. Smoker. Effects of hatchery releases and environmental variation on wild-stock productivity: consequences for hatchery enhancement of pink salmon in Prince William Sound, Alaska. Proc. Second International Symp. on Stock Enhancement and Sea Ranching. Kobe, Japan, Jan.28-Feb. 1, 2002. In press.

NMFS Involvement with Stock Enhancement as a Management Tool

Thomas D. McIlwain
U.S. Department of Commerce
National Oceanic and Atmospheric Administration
National Marine Fisheries Service, Mississippi Laboratories
3209 Frederic Street
Pascagoula, Mississippi 39567
USA
Email: Tom.McIlwain@noaa.gov

Abstract

During the last part of the 19th century and the first half of the 20th century the United States marine fishery management agency attempted to recover depleted fish stocks by propagating and releasing hatchery produced eggs and larvae. However, after over fifty years of effort, there was no evidence that this technique enhanced stocks. This was partly a result of the early approach to assessment, in which the numbers of fry produced determined success of hatchery programs rather than the number of adults that survived to enter the fishery. This early stock enhancement program was subsequently abandoned in favor of other techniques for recovering depleted fish stocks, such as restoring degraded nursery and spawning habitats and regulating fishing effort. In the 1940s and 1960s the United States Congress passed the Mitchell Act and the Anadromous Fish Act, which provided funds primarily to the U.S., with federal oversight, to culture and stock fish in an effort to rebuild depleted stocks of marine and anadromous fish. These fish stocking programs continue today. The National Marine Fisheries Service (NMFS, created in 1970) has traditionally used techniques other than stock enhancement as management tools to restore depleted stocks. However, over the past several years the U.S. Department of Commerce (DOC) and the National Oceanic and Atmospheric Administration (NOAA), the parent agency of the NMFS, have each adopted aquaculture policies that include stock enhancement. NOAA has also adopted a strategic plan to include stock enhancement.

The National Marine Fisheries Service (NMFS) and each of its predecessor agencies have been involved in aquaculture and stock enhancement for over 125 years. Marine fish culture methods developed during the second half of the nineteenth century were thought to augment and replenish natural stocks. In 1871 Spencer Fullerton Baird, leader of the newly formed U.S. Commission of Fish and Fisheries, reported to Congress the reasons for declining stocks and recommended fish culture as a solution (Baird, 1872). His ideas were accepted and a research vessel was built for the commission, followed by construction of shore-based marine fish hatcheries. Although highly efficient in producing and releasing fertilized eggs and newly hatched fry, the lack of evidence of increased harvest ended these efforts in the late 1940s. As the enhancement approach was abandoned the emphasis in post-war years shifted to "aquatic farming." Federal laboratories, led by the Bureau of Commercial Fisheries (now National Marine Fisheries Service, NMFS), conducted pioneer research in culture techniques, first in mollusks (at the Milford, Connecticut lab), then in salmonids (at the Manchester, Washington lab) and in marine shrimp (at the Galveston, Texas lab). Although these efforts were major contributions to new industries worldwide, federal

research in aquaculture and enhancement was drastically reduced in the 1980s. The agency generally abandoned stock enhancement as a tool to rebuild depleted stocks and concentrated on other management measures that included regulating fishing effort and restoring degraded nursery and spawning habitats. With a few exceptions these are still the primary management measures in place today.

Since NMFS does not utilize stock enhancement as a management tool Congress has chosen to continue its role by budgeting in support of stock enhancement programs to be conducted by groups other than NMFS. The United States has since become a leader in fish stock enhancement. This is principally due to a century of study with pacific salmon by the federal government and the states of the Pacific Northwest. The Columbia River Basin program is authorized and funded through the 1938 Mitchell Act as amended in 1946, which was established to mitigate, in perpetuity, for habitat and salmon runs lost through the construction of hydroelectric projects. Approximately $176 million was awarded to this program between 1970 and 1996. The 25 major hatcheries operated by this program release annually over 120 million smolts and contribute between 50-70% of all adults caught in the coastal fisheries of that region. McNair (2000) reported that 93.6% of all pink salmon caught in Prince William Sound in 1997 were from artificially propagated stocks. Of all salmon harvested in the common property resources in 1977 throughout Alaska, 22% of the coho salmon, 30% of the pink salmon, and 65% of the chum salmon originated from hatcheries. Fisheries enhancement through public, tribal, and non-profit (Alaska) propagation sites scattered along the length of the four western coastal states, contributes significantly to the domestic landings of pacific salmon, currently 285,147 metric tons valued at $270 million (NMFS, 2001), and to a large coastal recreational fishery.

Other federal legislation and financial support passed through the NMFS budget are aimed at restoring depleted stocks. These include the Anadromous Fish Act of 1965, which provides funds to the states to rebuild depleted stocks of anadromous fishes using enhancement techniques. The striped bass populations along the mid-Atlantic coast were at low population levels in the late 1960's. However, through cooperative efforts between the Atlantic States and NMFS these stocks have recovered today to a level that supports both a commercial and recreational fishery. Other efforts have been directed at reestablishing runs of sturgeon along the nation's coasts.

NMFS also assists aquaculture-related research and development through the Northeast Fishing Industry Grants Program. NMFS awarded $4.2 million to the program in 1994-1995 with the objective to help restore New England groundfish and shellfish stocks through hatchery programs, and to provide new business opportunities for displaced fishermen.

NMFS provides funds to the University of Southern Mississippi, which leads a consortium, dedicated to the development of marine enhancement techniques and involves Mote Marine Laboratory in Florida and the Oceanic Institute in Hawaii. Funding for that program totaled $2.5 million for fiscal year 2001. The Oceanic Institute has been awarded $0.5 million annually for several years to evaluate enhancement practices for several species of marine finfish.

In fiscal year 2002 Congress provided startup funds in the amount of $1.0 million for the Science Consortium for Ocean Replenishment and Enhancement (SCORE), which is a multi-state initiative for the recovery of the nation's ocean fisheries. Its approach is to replenish diminishing marine fisheries stocks based on scientific protocols developed through a highly coordinated, national effort and focused on the demonstration of successful stock enhancement. It is believed that this fast-track strategy has the potential to be more cost-effective and timely than policy measures traditionally used to conserve and sustain ocean fishery resources.

Enhancement practices are also being applied to the conservation of fish and shellfish

populations. The marine fisheries resources of the United States reached maximum production levels two decades ago. Modern catch trends show a high incidence of fish stocks that are either fully exploited, over exploited, depleted, or recovering. Some fishery managers believe that enhancement is an effective solution for replenishment and recovery and may be the only option for some species listed under the Endangered Species Act. Perhaps the most successful project has been the federal fisheries scientists' rescue and rehabilitation of the Redfish Lake sockeye salmon from the verge of extinction. Another may be the cooperative project by the United States and Mexico to save the Kemp's Ridley sea turtle.

In the last several years DOC/NOAA, parent organizations of NMFS, have both adopted policies on aquaculture that include stock enhancement as a fishery management tool.

Literature Cited

Baird, S. F. 1873. United States Commission of Fish and Fisheries. Report of the Commissioner for 1871.
McNair, M. 2000. Annual report. Alaska Department of Fish and Game. Juneau, AK. 34p.
NMFS. 2001. Fisheries of the United States. Current Fishery Statistics No. 2000. August 2001.

Stock Enhancement Research with Anadromous and Marine Fishes in South Carolina

Theodore I. J. Smith
Wallace E. Jenkins
Michael R. Denson
Mark R. Collins
Marine Resources Research Institute, South Carolina Department of Natural Resources
P. O. Box 12559
Charleston, South Carolina 29422
USA
Email: smitht@mrd.dnr.state.sc.us

Abstract

South Carolina's Marine Resources Research Institute (Department of Natural Resources) has been active in stock enhancement research since 1985. Initial efforts focused on a semi-anadromous endangered species, shortnose sturgeon *Acipenser brevirostrum*. Spawning, hatchery, and rearing techniques were developed using broodstock acquired from the Savannah River (the boundary river between South Carolina and Georgia), and over an 8-year period nearly 100,000 progeny (18.7% of which were tagged) were released back into that river. Stocking site, season, and fish sizes were varied in order to develop an optimal stocking protocol, and a number of studies were conducted to determine the best tagging method(s). A recent study designed to evaluate the success of the stocking program indicated that 38.7% of the adult population in the Savannah River is now comprised of stocked fish, and that the stocked fish have matured and are participating in spawning migrations. However, while wild fish generally do not leave their natal river, identifiable stocked fish have been captured in several other systems up to 278 km away from the mouth of the Savannah River. Data indicate that these wandering fish were almost exclusively individuals stocked at advanced ages and thus they may not have imprinted on the target river.

Since 1988, stock enhancement research has been conducted on a recreationally important and overfished sciaenid species, red drum *Sciaenops ocellatus.* Photothermal conditioning permits precise control of tank spawning in any season. Stocking studies, which are ongoing, have been conducted in a number of estuaries. It has been verified that fish stocked at a size >100 mm TL do appear in angler creels when they achieve the minimum legal size, but that they are relatively expensive to produce. Less expensive early age 0 (~2 mo, 20-55 mm TL) fish are now being stocked. They can be effectively marked, stocked, and their dispersal pattern determined. They also appear in angler creels in large numbers. Optimal stocking density has been estimated, and up to 78% of the legal fish in study areas have been identified as stocked fish. Controlled stocking in certain index areas has produced strong evidence that wild fish are supplemented rather than replaced by stocked fish.

A recent (2001) stock enhancement research program has been initiated for cobia *Rachycentron canadum*. About 1,500 tagged juveniles (~5 months old) were released into Port Royal Sound, SC, the estuary from which the broodfish were obtained. These were the first cobia stocked on the east coast of the U.S. This program has generated a great deal of interest from local angling clubs and fishing guides, and a number of these groups have provided labor and/or funds to assist the project. Near-term plans for research on this species include refinement of spawning methodology and growout techniques, as well as additional stocking activities. to elucidate early life history characteristics.

Introduction

South Carolina (SC) has been a pioneer in marine and estuarine stock enhancement research. Significant regional involvement dates to the mid-1960's and was focused on production of striped bass *Morone saxatilis* and its hybrids (especially white bass *M. chrysops* hybrids). The work was performed by staff of the Wildlife and Freshwater Fisheries Division of the South Carolina Department of Natural Resources (SCDNR). Development of a hormone based technique for spawning striped bass was a major breakthrough (Stevens, 1967) and formed the foundation for expansion of production activities. Although originally stocked to control gizzard shad *Dorosoma cepedianum*, the striped bass has become a premier freshwater game species. Based on the early work in South Carolina and neighboring states, striped bass and its hybrids are now stocked in estuaries, rivers, lakes and reservoirs throughout the United States (Bailey, 1975; Axon and Whitehurst, 1985).

Interest in stocking coastal rivers and estuaries began in 1985 with the establishment of a cooperative program between SCDNR and the United States Fish & Wildlife Service (USFWS). This program, conducted by the Marine Resources Research Institute (MRRI), Marine Resources Division, was broad based and examined the life history, ecology and potential for stock enhancement of the endangered shortnose sturgeon *Acipenser brevirostrum*. Stocking of this amphidromous fish occurred during the period 1984-1992, but funding for sampling to evaluate the success of the stocking program occurred only from 1990 to early 1993, and then again in 1997-2000. Fortunately, in the interim, other monitoring projects have provided substantial information on the occurrence and impacts of the stocked fish.

Decline in abundance of red drum *Sciaenops ocellatus* has been a major concern among fishery managers from the mid-Atlantic to the Gulf of Mexico for almost two decades. This species formerly supported substantial commercial and recreational fisheries from North Carolina (NC) through Texas (TX), and now all commercial fisheries have either been closed or severely restricted (Mercer, 1984; Matlock *et al.*, 1987; Goodyear, 1991; Wenner, 1992). Recreational anglers have also been impacted as evident by the increasingly restrictive size and creel limits that have evolved [1 fish/day in Florida (FL) and NC; 2 fish/day in SC]. In the most recent red drum virtual population analysis (VPA) the estimated escapement to spawning stock was 17% (Vaughan and Carmichael, 2000). This is an improvement over the previous estimate of 10% escapement in the 1993 VPA. However, even though escapement is slowly increasing, abundance of the population segment available to anglers (primarily subadults) appears to be declining, at least in SC (C. Wenner, SCDNR, Charleston, SC, personal communication). The current fisheries recruitment model suggests that a target escapement level of 40% is needed for a healthy population. Thus, it appears that full recovery of stocks through conventional management approaches will require a long time frame.

In 1988, MRRI initiated its first stock enhancement program with a marine species, red drum, to evaluate the use of hatchery fish as an additional management option. Initial stocking studies focused on use of larger juveniles which could be externally tagged. Results indicated that these fish survived and entered the creel of anglers as well as the adult population. Efforts then shifted to the use of smaller (~20-55 mm TL) juveniles that could be produced in larger numbers and at lower cost. These fish were marked with oxytetracycline-HCL (OTC) as well as genetically tagged. Using these smaller fish, it is now possible to stock 1–1.5 million/year as part of the research program, as compared to tens of thousands of larger fish.

During 2001, success in spawning and culturing cobia *Rachycentron canadum* has provided the opportunity to begin work with this highly migratory coastal species. This species, which has been reported to grow to a size of about 47 kg, inhabits most tropical, subtropical and warm temperate waters throughout the world (Shaffer and Nakamura, 1989). It is a prized game and valuable commercial species throughout its range. In spite of its high value as a seafood, landings are typically low and consist primarily of incidental captures. In the U.S. during 1998, combined landings from the Atlantic coast and Gulf of Mexico was approximately 150 mt with a value of about US $600,000 (Mills, 2000). Cobia inhabit SC waters during May to September (Bearden, 1961), but are absent during winter months. When here, they are the focus of specialty fishing, especially when aggregated for spawning. In conjunction with the local angling community, ~4-month old juveniles were tagged and stocked during fall 2001, around the time of their natural southern migration.

This manuscript provides a summary of the findings from the coastal stocking programs involving shortnose sturgeon, red drum and cobia.

Materials and Methods

General

The stocking programs were conducted under the auspices of MRRI. Stocking efforts were controlled as possible and performed in a fashion that has been described as the "responsible approach" by Blankenship and Leber (1995). In the case of the shortnose sturgeon, spawning activities took place at the Orangeburg National Fish Hatchery, SC (Smith, 1990; Smith and Jenkins, 1991; Smith *et al.*, 1993). Production of juveniles occurred at several USFWS hatcheries as well as MRRI's Charleston facilities and at the Waddell Mariculture Center (WMC), Bluffton, SC (Smith *et al.*, 1995). Red drum were spawned in Charleston and the juveniles produced in ponds at WMC (Smith *et al.*, 1999; 2001). Cobia spawning and production of juveniles occurred at WMC (Dodd, 2001).

Wild broodstock were used in all studies and were replaced during successive years of study. Released fish were marked and performance of marks/tags was documented. General health of fish was visually assessed before release. As possible, fish were held for several days to a week after marking to allow recovery before release into the wild. Samples from stocking groups were retained to assess tag retention or to validate biological marks, and to provide an estimate of post-stocking mortality. Tag recovery typically involved fishery independent as well as fishery dependent techniques. When external tags were used, tags contained reporting information as well as the offer of a reward.

Shortnose Sturgeon

The stocking research with shortnose sturgeon represents the first major effort in the U.S. with an acipenserid since the turn of the century. During 1984-1992, a state/federal program was conducted to evaluate the potential of enhancing/restoring populations of shortnose sturgeon through releases of hatchery reared fish (Smith and Jenkins, 1991; Smith *et al.*, 1995; Collins *et al.,* 1999). The Savannah River (Fig. 1) was chosen because ripe broodstock were obtainable but abundance of juveniles appeared low. A total of 97,483 sturgeon were stocked in the Savannah River. Of the fish released, 79,270 (81.3%) were untagged small juveniles (age 2-10 weeks, mean 41 mm TL, range 17-70 mm). They were stocked during 1984-1990 (except 1986). These fish were in excess of hatchery holding capabilities and were stocked in response to federal

directives. In addition, 18,213 juveniles were marked and released during 1986-1992 (except 1987). These fish varied substantially in size (mean lengths of different groups ranged from 76 to 762 mm TL) and ages (2.5 months to 3 years old). The largest and oldest fish were extras to a program focused on development of domesticated breeding stock. The types of marks used varied as did the longevity of retention and detection (Collins *et al.*, 1994; Smith *et al.*, 1990; Smith *et al.*, 2002a).

Program components included examination of various stocking issues including: season of stocking; area of stocking; size of fish at stocking; retention and impacts of various marks; and movement of stocked fish (Smith *et al.*, 1995; Smith and Collins, 1996). Broodstock were obtained from the bycatch of the commercial American shad *Alosa sapidissima* gill net fishery and from directed sampling with gill nets and trammel nets. Directed sampling for stocked fish ended in January 1993, shortly after the stocking activities

Figure 1. Map of Southeast United States showing locations where stocked shortnose sturgeon were captured.

were concluded. Directed sampling in the Savannah River resumed during 1997-2000 using gill and trammel nets. In the interim, bycatch data collected from the commercial American shad fishery was used to monitor the population.

During 1994-2000, gill net collections targeting sturgeons and American shad were conducted in the Edisto River, SC (Smith *et al.,* 2002b) (Fig. 1). Sampling was limited to river kilometer (rkm) 23 to 36, but due to the limitations of the gear (drifting gill net with varying mesh sizes), rkm 28 was the primary sampling area. The area of rkm 28 typically coincided with the vicinity of the saltwater/freshwater interface. Besides this directed effort, commercial American shad fishers in the Edisto River had been keeping records of incidental captures of sturgeons since 1979. Shad fishers reporting sturgeon captures in the Edisto and other SC river systems typically used gill nets with 13.3 cm stretch mesh webbing, which effectively captured adult shortnose sturgeon. Bycatch information was also provided by participants in other fisheries (e.g., commercial shrimp *Penaeus* spp. trawl fishery). Directed sampling for sturgeons was also conducted in the Ogeechee River, Georgia (GA) (Fig. 1). During 1993-1998 (excluding 1996), 46 m long trammel nets (7.6 cm stretch inner and 38.1 stretch outer mesh) were used, while in 1999-2000, 27 m long anchored gillnets of varying mesh sizes (1.9 to 8.9 cm stretch mesh) were fished.
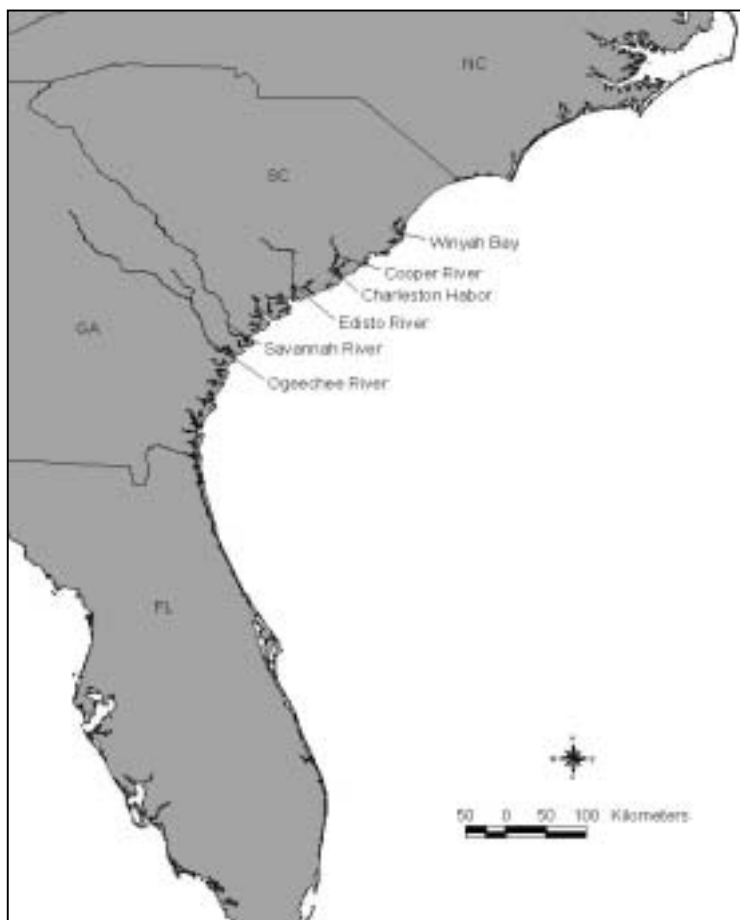
Red Drum

Research on red drum stock enhancement spans over a decade in SC. Initial work during 1988-1992 focused on identification of predictable spawning and juvenile production techniques and development of stocking protocols for juveniles (Smith *et al.*, 1992). Of substantial importance during this period was identification of tagging techniques for advanced juveniles and an evaluation of tag retention and reporting of tagged fish by anglers (Smith *et al.*, 1997; Jenkins *et al.*, 2000; Denson *et al.*, 2002). Evaluation of impacts was based on fishery independent (trammel netting) data coupled with fishery dependent (angler tag returns) data. During the mid-1990's, studies began to examine the impacts of stocking small red drum, as was being done in TX (Matlock, 1990; McEachron and Daniels, 1995; McEachron *et al.,* 1995; 1998). At this time, a chemical mark (OTC) was approved for use with red drum under an investigational new animal drug (INAD) permit issued by the U.S. Food and Drug Administration (FDA). Small fish could be more cheaply produced and thus more fish could be used in the stocking studies. Refinement of the marking technique was done and the retention of marks validated (Jenkins *et al.*, 2002). Small juveniles (20-45 mm TL) were produced in ponds out of season (May- June) and during the normal spawning season (August-October) and marked with OTC. After marking, fish were returned to a recovery pond for a week before stocking in Callawassie Creek, a tributary of the Colleton River and part of the Port Royal Sound estuary. This creek was located near the production facility. During 1995-1997, 1.7 million fish were released. Data on movements, contribution, growth, and sex composition were determined primarily from samples collected with trammel nets. Information obtained from trials conducted during spring 1995 are not included as marking, stocking, and sampling protocols were being developed during this period.

Current activities are examining the critical issue – "do stocked fish supplement a population or displace native fish." The Charleston Harbor estuary was selected for this study as there is an 8-year historical data base on red drum abundance based on a stratified random trammel net survey. Data were summarized for red drum less than 450 mm TL for nets set in 1 m depth or less water for the period July - December. Mean catch per unit effort (CPUE) for the Ashley River ranged from 0.3 to 1.4 fish/net set while the mean CPUE for the Wando River ranged from 1.2 to 8.4 fish/net set (Fig. 2).

These data support the hypothesis that the Ashley River has a much smaller population of age 1 red drum than does the Wando River. Due to the wide differences in the CPUE data, these two rivers were selected to examine the question of supplementation or displacement of wild fish by hatchery produced fish in a "degraded habitat" (Ashley River) and in a "healthy habitat" (Wando River). Fish were stocked over a 15 km reach of the river. The maximum potential nursery area was calculated over this distance using GIS technology. This calculation was made by adding the total coverage of *Spartina alterniflora* and the area of tributaries within the marsh while excluding the area covered by main river channel. Using this technique the stocked area of the Ashley River was estimated to contain a maximum of 980 ha of potential nursery area while the stocked area of the Wando River contained 2,903 ha. The Ashley River has been stocked for three years (1999-2001) with 617-687 fish/ha while the Wando River was stocked at a lower density of 118 and 177 fish/ha in 2001 and 2000, respectively (Table 1). Impacts are being evaluated based on recruitment of age 1 fish (~350 mm TL). Only results from the first year's stocking in the Ashley River are available.
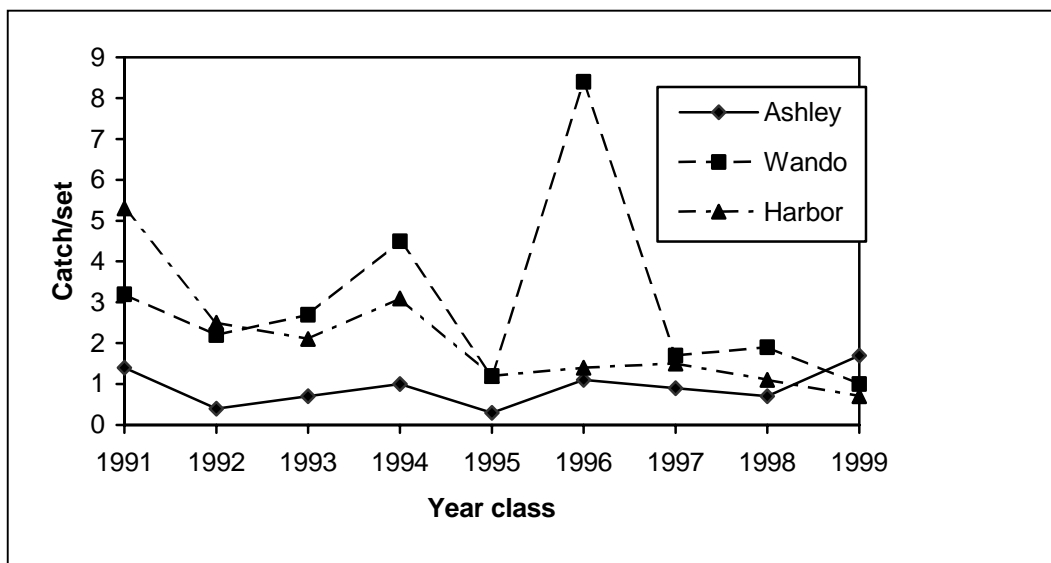
Figure 2. Random catch/unit effort data for age 11-16 month red drum collected in three areas of the Charleston Harbor estuary from each year class between 1991 and 1999. Hatchery fish (30 mm TL) were stocked in the Ashley River in the 1999 year class.

Table 1. Number released, mean total length and stocking density for red drum marked released each fall (Sept.- Nov.) in the Ashley and Wando Rivers between 1999 and 2001.

	Ashley River			Wando River		
Year	Number	Mean length (mm TL)	Density (n/ha)	Number	Mean length (mm TL)	Density (n/ha)
1999	617,190	30.0	630	--	--	--
2000	604,884	23.5	617	513,920	23.5	177
2001	673,751	20.8	687	344,949	20.4	118

Cobia

In the US, research with cobia has relied on field capture of wild fish as it has only been very recently that this species has been spawned in captivity and progeny produced (Sea Grant Virginia, 2000; Dodd, 2001; Arnold *et al.,* 2002). The spawning and culture successes in SC during 2001 produced advanced juveniles for research purposes. Interest in this species is three-fold: 1 – gain initial insights into the potential for stock enhancement; 2 – obtain basic

information on the movements, growth, recruitment, and homing instincts of the species; and, 3 – examine its potential for commercial aquaculture. Work in all these areas is underway. During fall 2001, a total of 1,523 fish were tagged and released in four locations in Port Royal Sound through the combined efforts of local angling clubs (Hilton Head Island Sportfishing Club; Beaufort Sportfishing and Dive Club) and project staff. The fish were tagged with a dart tag and stocked on October 9 and 24 at which time they were 130-144 days old (Table 2). Overall, fish had a mean size of 347 mm TL and weighed 331 g. These pond-reared juveniles were produced during the normal spawning season and were similar in size to wild juveniles. Due to the high level of interest and involvement of recreational anglers and fishing guides, this program has been publicized along the south Atlantic coast (e.g. Florida Sportsman, Hilton Head Island Packet).

Table 2. Mean size (range) of 1,523, ~4 month old cobia juveniles tagged (dart tag) and released in Port Royal Sound, SC in 2001.

Release date (mth/da/yr)	Mean length (mm TL)	Mean weight (g)	Released (#)
10/09/2001	341 (235-390)	309 (178-443)	881
10/24/2001	356 (294-402)	362 (193-533)	642

Results and Discussion

Shortnose Sturgeon

This federal/state program was the first effort to evaluate the use of hatchery produced shortnose sturgeon as a potential management tool for supplementation or restoration of this endangered species. Information collected during the stocking efforts in the Savannah River and shortly thereafter indicated that stocked juveniles comprised a minimum of 35.4% of the juvenile population in the lower river nursery area (Smith and Collins, 1996). Based on recovery of marked fish after a mean time out of 7.2 ± 1.9 years (range 5.9-10.4) and results from double tagging studies, it is estimated that at least 38.7% of the adult population in the Savannah River during 1997-2000 was made up of stocked fish (Smith *et al.*, 2002a) (Table 3).

Table 3. Contribution of hatchery produced shortnose sturgeon to the adult populations of three coastal rivers between 1990 and 2000. Tag loss/non-detection factor of 3.72 not applied to the known hatchery origin data (fish contained marks). Data are means with range shown in parenthesis.

River (state)	Years data collected	Known hatchery origin (%)	Time at large (yrs)	Mean size (TL cm)
Savannah (GA)	1997-2000	10.4	7.2 (5.9-7.9)	77 (63-96)
Edisto (SC)	1995-2000	10.6	6.7 (4.1-10.2)	78 (58-98)
Ogeechee (GA)	1997-2000	8.4	7.4 (5.4-9.7)	80 (75-95)

Further, it was documented that stocked fish contained mature gametes and participated in spawning migrations (Smith *et al.*, 2002a). Population estimates and CPUE data from 1997-2000 suggest that the adult population is now larger than 10 years ago, but juveniles are still rare. This suggests that a recruitment bottleneck exists during the early life stages. From field sampling data, water quality degradation in the nursery habitat is believed to be at least partially responsible for the poor recruitment in the Savannah River (Smith *et al.*, 2002a).

Data on capture of tagged fish obtained from directed sampling and from commercial fisheries bycatch in other river systems have provided additional information on the fate of the fish stocked in the Savannah River. Beginning in 1995, hatchery fish began to be captured in non-target rivers, especially the Ogeechee River, GA, and Edisto River, SC, the two large rivers closest to the Savannah River (Figure 1, Table 3). From 1997-2000, identifiable stocked fish comprised 8.4% of the adult population in the Ogeechee River, while from 1995-2000, 10.6% of the adults in the Edisto River were identifiable as stocked fish (Smith *et al.*, 2002b) (Table 3). These values, as well as that for the Savannah River, can also be expanded (multiplied by 3.72) based on tag loss data from double tagging studies. In the Edisto River there was a historical Atlantic sturgeon fishery and shad fishery, the latter of which continues today. However, no shortnose sturgeon had been reported from the Edisto River prior to 1994. It appears that fish stocked into the Savannah River emigrated and colonized the Edisto River, and in 1998 the first age 1 juvenile was captured. In the Ogeechee River it appears that substantial supplementation of the population has occurred from the fish stocked in the Savannah River. Other stocked fish have been detected in the Cooper River, SC and in Winyah Bay, SC, a maximum of 278 km from the mouth of the Savannah River (Smith *et al.*, in press b) (Figure 1).

This stocking research identified issues associated with long term marking of stocked fish and showed that marking of small juvenile sturgeon for later recapture as adults was especially problematic. Improvement in tagging technologies, including use of genetic markers, should help resolve this issue. Stocking protocols approximated current recommendations developed by the

Atlantic States Marine Fisheries Commission (ASMFC) for Atlantic sturgeon (ASMFC, 1996) and were used in part to help develop these recommendations. However, stocking similar numbers of progeny per mating was not well controlled. This, coupled with assumed differential survival of different size groups of stocked animals, may have genetic implications relative to other river systems (Quattro *et al.*, 2002). Future stocking efforts need to strive for balanced numbers of progeny per broodstock mating and similar survival rates. As straying of some stocked fish into non-target rivers was noted, future shortnose sturgeon (and perhaps other species) stocking programs should address the issue of imprinting. Perhaps straying into non-target areas can be reduced by raising fish in target river water or by stocking very young (assumed pre-imprinting) fish.

Red Drum

With the apparent limited success of conventional fishery management regulations in maintaining healthy red drum populations, SC and other states are implementing or examining the use of hatchery release programs as an additional management tool. Texas has developed a large scale program which it feels is highly effective, and stocks approximately 40 million small juveniles per year (McEachron *et al.*, 1998). South Carolina and FL (Willis *et al.*, 1995) are conducting controlled studies to evaluate this approach, while other states (e.g. NC – Copeland *et al.*, 1998; GA – Woodward, 2000) have an interest but are evaluating the success of neighboring states.

Studies conducted to date in SC have identified suitable protocols for release (size, season, habitat, etc.) and have documented the survival and growth of stocked fish for periods up to 4 years post-release (Smith *et al.*, 1997). Chemical treatments for batch marking large numbers of small fish, as well as the use of external tags (abdominal T anchor) for individually marking larger fish, have been shown to be suitable for use in large scale field programs. However, both tagging methods have limitations; OTC requires sacrifice of the fish while external tags require use of large fish. Although stocking of large fish in Charleston Harbor, SC has resulted in a contribution to the local population of up to 4.1% (Table 4), production and stocking costs are major considerations (Smith *et al.*, 1997).

Table 4. Annual contribution of externally marked hatchery fish to the wild red drum population in the Wando River, SC between 1989 and 1994 (size at release ranged from 100–300 mm TL). Samples were collected randomly using a trammel net and include all ages and sizes of red drum captured each year.

Year	Stocked (#)	Fish sampled (#)	Hatchery contribution (%)
1989	4,145	897	0.8
1990	5,961	784	3.8
1991	11,279	1,209	0.3
1992	15,409	2,265	4.1
1993	14,957	2,496	1.3
1994	0	2,246	1.0

A total of 1,574,862 red drum juveniles (mean length 22 - 56 mm TL) were marked with OTC and released in Callawassie Creek, Port Royal Sound estuary, from fall 1995 to spring

1997. Using various gear types, 1,687 fish from target size groups were captured and sacrificed. In addition, 1,722 larger fish were captured, measured and released. Controlled studies with captive marked fish indicated that the OTC marks were visible for at least 52 months (Jenkins *et al.*, 2002). Results of analyses of otoliths indicated that there were no differences in growth rates of wild fish before or during the years that stocking occurred. Hatchery fish released in the fall grew at a rate similar to wild fish of the same year class (Smith *et al.*, 1999). Impacts of the stocked fish were evaluated over a 30 km distance from the stocking site. Sampling in the Port Royal Sound estuary of age 0 - 3 fish from the 1995 and 1996 year classes indicated that overall, hatchery fish comprised 19.4% (n=252) of the 1,316 fish collected from these year classes. Contribution to the local population at the release site increased as stocking number increased, with a maximum of 77.3% hatchery contribution recorded for the 1996 year class at Callawassie Creek, the stocking site (Fig. 3). However, in the estuary as a whole this proportional increase was not sustained, as the intermediate stocking number of 346,926 fish (1995) provided an overall contribution of 19.0%, which is similar to that recorded (19.7%) for the highest release number (1.2 million). Hatchery contribution decreased as fish aged, and fall hatchery fish (natural spawning time) appeared to make a larger proportional contribution than fish released out of season in the spring (Smith *et al.*, 1999). Hatchery and wild fish of various ages occupied similar habitats and were frequently captured in the same net sets. In addition, wild and hatchery-produced fish exhibited similar sex ratios (Smith *et al.*, 1999).
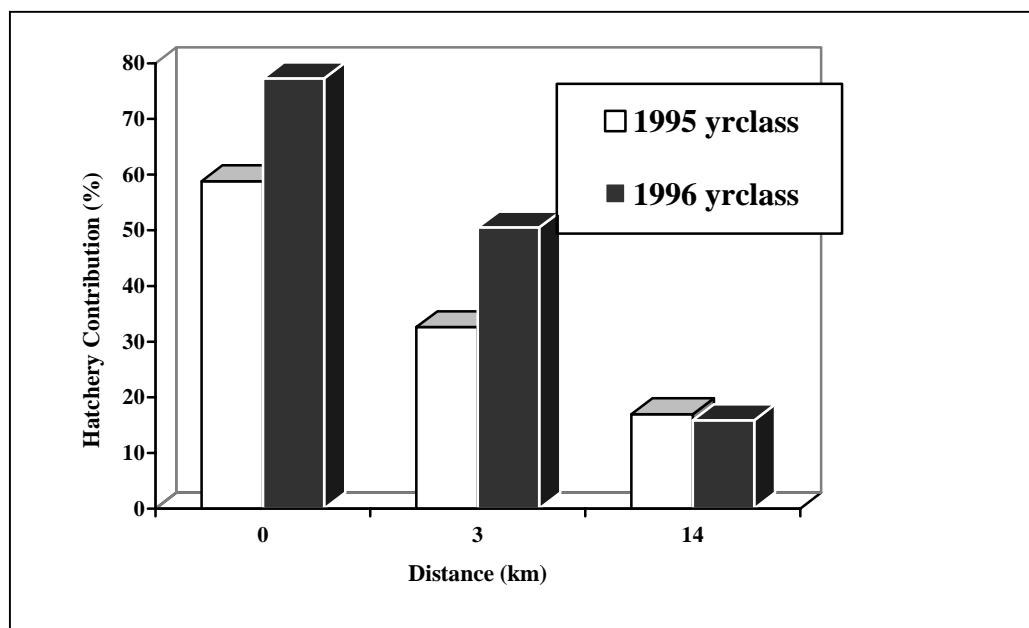

Figure 3. Percentage of all fish captured from each year class at each site between Summer 1996 and Spring 1999 which were of hatchery origin. The primary sample sites were the release site (0 km) Callawassie Creek; Rose Hill flats (3 km from release site) and the Chechessee River (14 km from release site).

The current phase of research is focused on the critical issue of supplementation vs. displacement. Stocked fish begin to recruit to the trammel nets used to sample the population at

about 10 months of age, so only results from the 1999 stocking in the Ashley river are available. During December 2000 - February 2001, fish were taken (sacrificed) from the Ashley River to determine the percent contribution of stocked fish, as well as from Charleston Harbor and the Wando River to see if any movement had occurred. Based on OTC marked otoliths, stocking has had a significant supplemental impact on the Ashley River population (78% contribution) (Table 5) with the CPUE exceeding the highest level on record for this river (Table 6; Fig. 2). In fact, the Ashley River CPUE was the highest reported from any sampling site in the entire state, further demonstrating the depressed level of red drum stocks (Table 6). Besides the substantial impact on fish abundance in the Ashley River, a substantial portion of the fish in the Charleston Harbor estuary and a smaller portion of the fish in the Wando River were identified as originating from fish stocked in the Ashley River (Table 5).

Table 5. Hatchery contribution (%) to the 1999 year class of red drum as determined by both fishery independent and dependent sampling in the Ashley and Wando Rivers and Charleston Harbor. A total of 617,190 juvenile red drum were stocked in the Ashley River only.

Site	Fishery Independent		Fishery Dependent	
	Number sampled	Hatchery (%)	Number Sampled	Hatchery (%)
Ashley River	41	78	20	70
Wando River	51	12	3	0
Charleston Harbor	20	15	25	52

Table 6. Catch per unit effort in descending order for young of year red drum collected during random trammel net sampling along the coast of SC between July and December 2000.

Site	Effort (net sets)	Mean CPUE	Standard Deviation
Ashley River	41	1.73	3.7
Ace Basin	41	1.34	3.6
Muddy Bay	45	1.07	1.7
Wando River	32	1.00	2.4
Charleston Harbor	38	0.74	1.0
Romaine Harbor	46	0.63	1.3

These findings are quite dramatic and could have far reaching implications for SC and other states exploring the possibility of hatchery releases as a fishery management tool. However, data are from only the first year of this multi-year project and thus should be considered as preliminary. Additional annual stocking and data collection efforts in the Ashley and Wando Rivers will allow a time sequence of replication over which impacts can be assessed. This issue of supplementation vs. displacement has been identified as a priority concern and needs to be quantitatively addressed (Hilborn, 1999).

Cobia

There is limited information on the impacts of the stocked cobia in this new program. However, within a week of both stockings, there were anecdotal reports of a captured fish within the general release area. One fish was reported by an angler who was fishing in a tournament. He caught a tagged juvenile by hook and line but the fish was lost overboard before the tag details could be obtained. The second report came from a recreational shrimp baiter. While cast netting for shrimp at night he caught a cobia of the general size that had been stocked. No tag was noted before the fish was released. Both reports came from Port Royal Sound. A third fish was reported as being captured on October 27, 2001 in the surf at Cocoa Beach FL. This fish was from the October 9th release and had traveled approximately 480 km south during 18 days at large (26.7 km/day).

It is anticipated that during winter 2002 additional captures will be reported, as there is a suspected north-south movement pattern during fall along the southeastern U.S. (Hardy, 1978). In spring, fishermen have tracked the movement of adult cobia in the opposite direction (McNally, 1985). There is also a report of a wild cobia tagged off Charleston in June 1984 being recaptured off Biloxi, Mississippi in April 1986 (Shaffer and Nakamura, 1989). In SC, there have also been several reports of juveniles being captured in offshore waters during winter months suggesting an offshore movement (D. Hammond, SCDNR, Charleston, SC).

Little information exists on the population abundance of cobia. Recent VPA's on fish abundance in the Gulf of Mexico and along the Atlantic coast have been inconclusive due to a lack of sound modeling data (D. Hammond, SCDNR, Charleston, SC, personal communication 2001). However, it is felt that populations in the Gulf and along the south Atlantic have increased since the early 1980's. Information obtained from the stocking effort will provide new insights into the movements, growth, and potential for stock enhancement of this species.

Conclusions

South Carolina has made substantial progress in evaluating the utility of stock enhancement as a management tool. Although additional information is needed, the results to date suggest that release of hatchery fish may benefit depleted stocks. However, all efforts should be conducted in a responsible manner and stocking of hatchery fish should not be viewed as a substitute for strict habitat protection. Further, stock supplementation efforts should be coupled with conventional conservation/management actions to maintain the long term health of the populations.

Acknowledgments

Literature Cited

Arnold, C. R., J. B. Kaiser, and G. J. Holt. Spawning of cobia (*Rachycentron canadum*) in captivity. *J. World Aqua. Soc.* in press.

Atlantic States Marine Fisheries Commission. 1996. Fisheries Management Report No. 68. Breeding and stocking protocol for cultured Atlantic sturgeon. Washington, D.C. 19 pp.

Axon, J. R. and D. K. Whitehurst. 1985. Striped bass management in lakes with emphasis on management problems. *Trans. Amer. Fish. Soc.* 114:8-11.

Bailey, W. M. 1975. An evaluation of striped bass introductions in the southeastern United States. Proceedings Southeast Association of Game and Fish Commissioners 28:54-68.

Bearden, C. M. 1961. Common Marine Fishes of South Carolina. Contributions of the Bears Bluff Laboratory 34. Charleston, SC. 47 pp.

Blankenship H. L. and K. M. Leber. 1995. A responsible approach to marine stock enhancement, pp. 167-175 In*: H. L. Schramm and R. G. Piper (eds.) Uses and Effects of Cultured Fishes in Aquatic Ecosystems, American Fisheries Society. Bethesda, MD.

Collins, M. R., T. I. J. Smith, and L. D. Heyward. 1994. Effectiveness of six methods for marking juvenile shortnose sturgeon. *Prog. Fish-Cult.* 56:250-254.

Collins, M. R., T. I. J. Smith, K. Ware, and J. Quattro. 1999. Culture and stock enhancement of shortnose and Atlantic sturgeons. *Bull. Nat. Inst. Aqua.* (Japan), Supplement 1:101-109.

Copeland B. J., J. Miller, and E.B. Waters. 1998. Potential for Flounder and Red Drum Stock Enhancement in North Carolina. North Carolina Sea Grant Program. Raleigh, NC. 22pp.

Denson, M. R., W. E. Jenkins, A. G. Woodward, and T. I. J. Smith. 2002. Sport angler's reporting of tagged estuarine dependant fish in South Carolina and Georgia. *Fish. Bull.* 100:35-41.

Dodd. Q. 2001. U S cobia culture meets early success. *Hatch. Internat.* 2(4):14-17.

Goodyear, C. P. 1991. Status of the red drum stocks in the Gulf of Mexico. NMFS Southeast Fisheries Center Costal Resource Division Contribution MIA-90/91-87. Miami, FL.

Hardy, J. D., Jr. 1978. Development of fishes of the Mid Atlantic Bight. An atlas of egg larval, and juvenile stages. Vol. III. Aphredoderidae through Rachycentridae. USFWS, Biological Services Program, FWS/OBS-78/12. 394 pp.

Hilborn, R. 1999. Confessions of a reformed hatchery basher. *Fisheries* 24(5):30-31.

Jenkins, W. E., M. R. Denson, C. B. Bridgham, Mark R. Collins, and Theodore I. J. Smith. 2002. Retention of oxytetracycline induced marks on sagittae of red drum. *N. Amer. J. Fish. Mngt.*, 22:590-594.

Jenkins, W. E., M. R. Denson, and T. I. J. Smith. 2000. Determination of angler reporting level for red drum (*Sciaenops ocellatus*) in a South Carolina estuary. *Fish. Res.* 44:273-277.

Matlock, G. C. 1990. Preliminary results of red drum stocking in Texas, pp. 11-15. In*:* A. K. Sparks (ed.). Marine Farming and Enhancement: Proceedings of the 15th U.S. Japan Meeting on Aquaculture. NOAA Technical Report NMFS 85. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service. Washington, D.C.

Matlock, G. C., R. L. Colura, A. F. Maciorowski, and L. W. McEachron. 1987. Use of on going tagging programs to validate scale readings, pp. 279-285. In: R. C. Summerfelt (ed). The Age and Growth of Fish. Iowa State University Press. Ames, IA.

McEachron, L. W., R. L. Colura, B. W. Bumguardner, and R. Ward. 1998. Survival of stocked red drum in Texas. *Bull. Mar. Sci.* 62:359-368.

McEachron, L. W. and K. Daniels. 1995. Red drum in Texas: A success story in partnership and commitment. *Fisheries* 20(3):6-8.

McEachron, L. W., C. E. McCarty, and R. R. Vega. 1995. Beneficial uses of marine fish hatcheries: enhancement of red drum in Texas coastal waters. *Amer. Fish. Soc. Symp.* 15:161-166.

McNally, B. 1985. Run to the rays. *Florida Sportsman* 16(6):33-36.

Mercer, L. P. 1984. Fishery management plan for the red drum (*Sciaenops ocellatus*) fishery. North Carolina Department of Natural Resources and Community Development. Morehead City, NC. 105 pp.

Mills, S. 2000. A cobia by any other name. *Virginia Marine Resources Bulletin* 32(1):2-10.

Quattro, J. M., T. W. Greig, D. K. Coykendall, B. W. Bowen, and J. D. Baldwin. 2002. Genetic issues in aquatic species management: the shortnose sturgeon (*Acipenser brevirostrum*) in the southeastern United States. *Conserv. Genetics*, 3(2):155-166.

Sea Grant Virginia. 2000. VIMS scientists successfully spawn cobia. *The Crest* 2(2):1-7.

Shaffer, R.V. and E. L. Nakamura. 1989. Synopsis of biological data on the cobia *Rachycentron canadum* (Pisces: Rachycentridae) NOAA Technical Report NMFS 82 (FAO Symposium 153). 21 pp.

Smith, T. I. J. 1990. Culture of North American sturgeons for fishery enhancement, pp. 19-27 In*:* A. K. Sparks (ed). Marine Farming and Enhancement: Proceedings of the 15th U.S. Japan Meeting on Aquaculture. NOAA Technical Report NMFS 85. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service. Washington, D.C.

Smith, T. I. J. and M. R. Collins. 1996. Shortnose sturgeon stocking success in the Savannah River. Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies 50:112-121.

Smith, T. I. J., M. R. Collins, W. C. Post, and J. W. McCord. 2002a. Stock enhancement of shortnose sturgeon: a case study. American Fishery Society Symposium. Bethesda, MD, 28:31-44.

Smith, T. I. J., M. R. Collins, and E. Kennedy. 1993. Identification of critical habitat requirements of shortnose sturgeon in South Carolina. Final Report Project AFS-17, USFWS. Atlanta, GA. 97 pp.

Smith, T. I. J. and W. E. Jenkins. 1991. Development of a shortnose sturgeon, *Acipenser brevirostrum*, stock enhancement program in North America, pp. 329-336. In: P. Williot (ed.). Acipenser. Centre National du Machinisme Agricole du Genie Rural des Eaux et des Forets.

Smith, T. I. J., W. E. Jenkins, and M. R. Denson. 1997. Overview of an experimental stock enhancement program for red drum in South Carolina. *Bull. Nat. Res. Inst. Aqua. Suppl.* 3:109-115.

Smith, T. I. J., S. D. Lamprecht, and J. W. Hall. 1990. Evaluation of tagging techniques for shortnose and Atlantic sturgeon. *Amer. Fish. Soc. Symp.* 7:134-141.

Smith, T. I. J., L. D. Heyward, W. E. Jenkins, and M. R. Collins. 1995. Culture and stock enhancement of shortnose sturgeon, *Acipenser brevirostrum*, in the southern United States, pp. 204-214. In*:* A. D. Gershanovich and T. I. J. Smith (eds). Proceedings of the second international symposium on sturgeons, VNIRO Publishing, Moscow, Russia.

Smith. T. I. J., W. E. Jenkins, M. H. Glenn, and D. B. White. 1992. Evaluation of a preliminary red drum stock enhancement program in South Carolina. Final Report Project F-34. USFWS. Atlanta, GA. 97 pp.

Smith, T. I. J., J. W. McCord, M. R. Collins, and W. C. Post. 2002b. Stock enhancement of shortnose sturgeon, *Acipenser brevirostrum*: occurrence in non-target rivers. Proceedings 4th International Conference on Sturgeon. July 2001 Oshkosh, WI.

Smith, T. I. J., W. E. Jenkins, M.R. Denson, C. B. Bridgham, and R. W. Chapman. 2001. Effects of stocking red drum in areas of low and high CPUE. Annual Report Project F-71. USFWS, Atlanta, GA. 13 pp.

Smith, T. I. J., W. E. Jenkins, M. R. Denson, C. B. Bridgham, and R. W. Chapman. 1999. Use of tidal creeks by biologically marked and wild juvenile red drum. Final Report, Project F-65. USFWS, Atlanta, GA. 42 pp.

Stevens, R. E. 1967. A final report on use of hormones to ovulate striped bass *Roccus saxatilis* (Walbaum). Proceedings of the Southeastern Association of Game and Fish Commissioners. 18:525-538.

Vaughan, D. S. and J. T. Carmichael. 2000. Assessment of Atlantic red drum for 1999: northern and southern regions. NOAA Technical Memorandum NMFS-SEFSC-447.

Wenner, C. A. 1992. Red drum natural history and fishing techniques in South Carolina. South Carolina Wildlife and Marine Resources Department, Educational Report 17. Charleston, SC. 40 pp.

Willis, S. A., W. W. Falls, C. W. Dennis, D. E. Roberts, and P. G. Whitchurch. 1995. Assessment of season of release and size at release on recapture rates of hatchery reared red drum, pp. 354-365. In*:* H. L. Schramm and R. G. Piper, (eds.). Uses and Effects of Cultured Fishes in Aquatic Ecosystems, American Fisheries Society Symposium 15. Bethesda, MD.

Woodward A. G. 2000. Red Drum Stock Enhancement in Georgia: A Responsible Approach. Coastal Resources Division, Georgia Department of Natural Resources, Brunswick, GA. 12 pp.

Comparison of Some Developmental, Nutritional, Behavioral, and Health Factors Relevant to Stocking of Striped Mullet (Mugilidae), Sheepshead (Sparidae), Common Snook (Centropomidae), and Nassau Groupers (Serranidae)

John W. Tucker, Jr.
Sarah Blain Kennedy
Division of Marine Science
Harbor Branch Oceanographic Institution
5600 North U.S. One
Fort Pierce, Florida 34946
USA
Email: tucker@hboi.edu

Striped mullet (*Mugil cephalus*, family Mugilidae) are temperate to tropical (Table 1), euryhaline (Table 2), schooling, very omnivorous, coastal fish that eat detritus and a wide range of other organic material. Sheepshead (*Archosargus probatocephalus*, family Sparidae) are temperate to tropical, euryhaline, territorial, omnivorous, coastal fish that are more specialized in feeding; crustaceans and mollusks are important in their diet. Common snook (*Centropomus undecimalis*, family Centropomidae) are tropical, euryhaline, schooling, carnivorous, coastal fish that eat mainly fish and crustaceans. Nassau groupers (*Epinephelus striatus*, family Serranidae) are tropical to temperate, moderately stenohaline, territorial, carnivorous, reef fish that eat mainly fish, crustaceans, and molluscs. We (Tucker, 1998) have raised these species to ages of more than 7, 13, 15, and 15 years (mullet, grouper, sheepshead, snook, respectively).

Table 1. Suitable temperatures (°C).

Species	Spawning	Larvae	Juveniles
Striped mullet	23	26	28
Sheepshead	23	27	28
Common snook	26	27	28
Nassau grouper	26	27	30

Table 2. Suitable salinities (ppt).

Species	Spawning	Best for larvae	Range for larvae	Best for juveniles	Range for juveniles
Striped mullet	32	26	17-36	⊦	0-55
Common snook	32	32	⊦	0	0-35
Sheepshead	35	⊦	15-36	⊦	<1-44
Nassau grouper	35	32	27-38	30	15-37

In Florida, striped mullet spawn offshore during December-February, sheepshead nearshore during March-April, common snook nearshore and in bays during April-October, and Nassau groupers on reefs during March-April. Nassau groupers spawn during the winter in the Caribbean region and summer at Bermuda.

Eggs and larvae are planktonic. Striped mullet eggs are ~940 µm in diameter and hatch in ~34 h at 24! C. They become juveniles 35-45 mm TL at ~28 days after hatching (dah). Sheepshead eggs are ~820 µm in diameter and hatch in ~28 h at 23! C. They become juveniles 14-20 mm TL at ~39 dah. Common snook eggs are ~750 µm in diameter and hatch in ~17 h at 28! C. They become juveniles 35-40 mm TL at ~35 dah. The life history of snook is very similar to that of barramundi (*Lates calcarifer*), except for later transformation (17-25 mm TL at 25 dah for barramundi). Nassau grouper eggs are ~1 mm in diameter and hatch in ~26 h at 26! C. They become juveniles 35-50 mm TL at 46-70 dah. The best reported survival in hatchery tanks has been 5.0% for Nassau grouper (egg-98 dah), 7.0% for common snook (hatchling-55 dah), 39.7% for sheepshead (egg-100 dah), and 52% for striped mullet (hatchling-60 dah).

Juveniles of all but Nassau grouper will thrive in hard fresh water. Nassau groupers typically are reef fish (Table 3). We used floating milk crates to accelerate transformation of larvae to juveniles in the hatchery. The others are coastal fish but can be found on islands with freshwater rivers. From the early juvenile stage, striped mullet are strictly schooling fish. Common snook form loose schools. Nassau groupers school as juveniles but become territorial as they mature. Sheepshead are territorial from the juvenile stage onward.

Table 3. Typical habitats.

Species	Larvae	Juveniles	Adults
Striped mullet	Coastal offshore	Shallow coastal	Shallow coastal & estuaries
Sheepshead	Coastal offshore	Structures, grassbeds	Structures, grassbeds
Common snook	Coastal offshore	Shallow vegetation	Shallow coastal & estuaries
Nassau grouper	Deep offshore	Backreef	Forereef

Striped mullet are extremely active omnivores (small foods, including detritus), almost constantly swimming and feeding over wide areas. Sheepshead are less active, spending long periods feeding over smaller areas, mainly on crustaceans, mollusks, and some algae. Common snook are less active, with short intense feeding periods on fish and crustaceans. They mostly hover and save energy when not feeding. Nassau groupers are least active and relatively sedentary, with short feeding periods, often opportunistic, on fish, crustaceans, and molluscs.

Striped mullet are especially susceptible to ectoparasites and scale loss (from handling) leading to vibriosis. Common snook are resistant to parasites and other diseases unless they are handled, in which case they become more susceptible to scale loss and vibriosis. Nassau groupers are susceptible to some ectoparasites like monogenean flatworms, but are very resistant to handling. Sheepshead are very resistant to diseases and handling. All of these species except groupers can be stocked in fresh water to avoid vibriosis and some parasites, but stocking in salt water can help avoid fungal infections.

Since 1996, we have used probiotic bacteria combined with improved environmental control to enhance health, survival, and growth of larvae of several species (Kennedy *et al.,* 1998). One impediment to snook farming has been the need for refining larval culture techniques to increase survival, especially during the first week. When rearing water was inoculated with *Bacillus* species isolated from healthy hatchery-reared snook, survival from hatching to 55-day-old juveniles was increased from the 1-2% reported previously to 7.0%. It is expected that effective control of cannibalism will further increase survival to at least 10-15% (tens of thousands times higher than in nature). With consistent egg quality and management, this level of survival would make large-scale rearing of snook feasible.

For stocking in grow-out tanks or small protected ponds and cages, fully developed juveniles ~50 mm TL are a good minimum. For less protected ponds and cages, the fish should be larger. For stocking in natural environments, the larger the fish the better. It is important to stock them at a size beyond which habitat (Table 3) and/or food scarcity are not limiting. As snook grow from 25 mm to 250 mm TL, they depend on shelter from shallow weeds, then mangroves, then grassbeds (or equivalents). As Nassau groupers grow from 50 mm to 250 mm TL, they move from shallow backreefs to shallow forereefs. Sheepshead live close to or in natural or artificial structures from the early juvenile stage onward. Mullet tend to stay in large schools in open water (shallow and deep) from the early juvenile stage onward.

Much behavior in these fish is innate. Striped mullet are foragers, always ready to flee. Sheepshead are grazers, not easily disturbed. Common snook are raptors, alert and suspicious of any movement or change in light. Nassau groupers are ambushers, bold and curious around humans unless mistreated. As they transform, reared striped mullet swim around the tanks in a school as if migrating. They seem just as fearful as wild mullet and thoroughly scour the tank walls and bottoms for food. When released in a shallow estuary, mullet swam away in a school. Once common snook are juveniles, they are almost as shy as mullet but stay near the bottom except when feeding. When released in shallow estuaries, snook swam away in small groups, hid between mangrove roots, and soon began chasing prey-size wild fish. Once sheepshead are juveniles with well-developed incisors, they thoroughly scrape algae from the tank walls. When released in a shallow estuary, they individually swam between mangrove roots and soon began scraping food from the roots. Nassau groupers are very adaptable during rearing, and will even eat directly from a person's hand immediately after being moved between tanks. Wild juveniles ate pellets from our hands within 2 h after capture. After being raised on dry pellets for 2 years, juveniles showed no hesitation in eating live goldfish, minnows, and fiddler crabs, and needed only a few hours to eat live shrimp when first
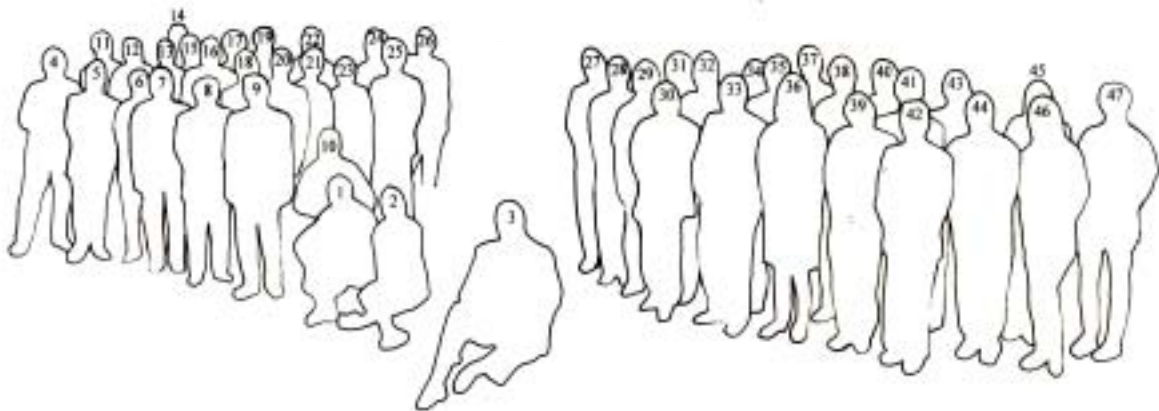
offered. At about the same time, several of them learned to knock large isopods off the tank wall several cm above the water by spitting streams of water like archerfish, and then ate them. When released on a 15-m reef after spending several days in a holding cage (Roberts *et al.*, 1995), reared Nassau groupers immediately went to a cleaning station to have parasites acquired in the cage removed from their mouths and gills by cleaner gobies and shrimp (like wild groupers). They soon settled into the reef, and within 2 d hunted alongside a moray eel and an octopus (like wild groupers).

Preconditioning can help released fish adapt more quickly. However, the swimming, feeding, social, and fright behaviors of these four species when reared are surprisingly similar to those of wild fish. In addition to providing biological needs and minimizing predation, good choice of release habitat can help limit stress and disease.

Literature Cited

Kennedy, S. B., J. W. Tucker, Jr., C. L. Neidig, G. K. Vermeer, V. R. Cooper, J. L. Jarrell, and D. G. Sennett. 1998. Bacterial management strategies for stock enhancement of warmwater marine fish: a case study with common snook (*Centropomus undecimalis*). *Bull. Mar. Sci.* 62:573-588.

Roberts, C. M., N. Quinn, J. W. Tucker, Jr., and P. N. Woodward. 1995. Introduction of hatchery-reared Nassau grouper to a coral reef environment. *N. Amer. J. Fish. Mgt.* 15:159-164.

Tucker, J. W., Jr. 1998. Marine fish culture. Kluwer Academic Publishers, Boston, x + 750 p.

■ Participants in the Thirtieth U.S. – Japan Meeting on Aquaculture

Identification List

1. Harry Grier
2. Kerri Duchon-Allard
3. Ken Leber
4. John Miller
5. Kazumasa Ikuta
6. Robert Iwamoto
7. Thomas Jamir
8. Anthony Calabrese
9. Yasuaki Nakamura
10. Charles Helsley
11. Makoto Yamasaki
12. Ted Smith
13. Kazuya Nagasawa
14. Robert Chapman
15. Dominic Preiswerk
16. Paul Kilho Park
17. Reginald Blaylock
18. Toyomitsu Horii
19. Nathan Brennan
20. James McVey
21. Eileen McVey
22. Greg Vermeer
23. M.-Seok Gwak
24. John Ransier
25. Masashi Sekino
26. David Ziemann
27. William Heard
28. Kunikazu Shimamoto
29. John Tucker
30. Tetsuo Seki
31. Woo-Seok Gwak
32. Ronald Goldberg
33. James Sullivan
34. Mark Drawbridge
35. Tom McIlwain
36. Linda Chaves
37. Michael Tringali
38. Conrad Mahnken
39. Osamu Tominaga
40. Gary Pruder
41. Tetsuo Fujii
42. Takeshi Murai
43. William Halstead
44. Toshihiro Shigeta
45. Kerri Mesner
46. Gina Russo
47. Cynthia Armstrong