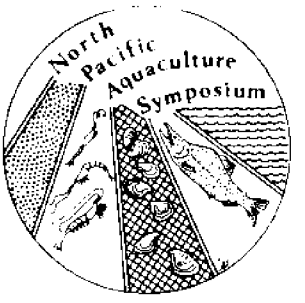

Proceedings of the North Pacific Aquaculture Symposium

Anchorage, Alaska
August 18-21, 1980

Newport, Oregon
August 25-27, 1980

University of Alaska

Alaska Sea Grant Report 82-2 January 1982

Alaska Sea Grant College Program
University of Alaska
Fairbanks, Alaska 99701

Proceedings of the North Pacific Aquaculture Symposium

University of Alaska, U.S.A.

Ministry of Fisheries, U.S.S.R.

Tokai University, Japan

Fisheries and Oceans, Canada

Brenda R. Melteff
Richard A. Nevé
Editors

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Acknowledgements

The North Pacific Aquaculture Symposium was sponsored by the University of Alaska Sea Grant College Program, the University of Washington Sea Grant College Program, Oregon State University Sea Grant College Program, Pacific Sea Grant Advisory Programs, Oregon Aqua-Foods, Inc., and Tavolek Inc.

We wish to thank all the people who made the many field trips taken during the symposium enjoyable and educational. The time we spent together at the various locations not only brought the delegates closer together, but allowed many others to contribute to the symposium and develop an international rapport among Pacific aquaculturists which will serve us well in the future.

In particular, we wish to thank the following people.

Alaska Department of Fish and Game personnel Loren Flagg, Dave Waite, Jeff Koenings, Nick Dudiak, Alan Quimby, Ken Tarbox and Dave Waltemeyer for their presentations at the sonar counting station, the demonstration of the coded wire tag procedures and general overview of their department's projects, and Dave Daisy, Bill Hauser and Paul Krasnowski who spent many extra hours organizing these trips.

Conrad Mahnken of the National Marine Fisheries Service for his time and energy in giving a tour of the Manchester Facility, and Ted Brewer and Jon Lindbergh for the excellent movies and discussion concerning the activities of Domsea, Inc.

For the very enjoyable tour of the University of Washington Big Beef Creek facility we thank Dr. Ernie Salo, Bruce Synder and the staff of the facility. A special thanks to students Steve Shroder and Gary Duker for their excellent presentations on their research.

Bob Hagar organized a tour of the George Adams and McKernan hatcheries and supplied us with wonderful picnic grounds.

The tour of the Whiskey Creek Oyster Farms conducted by Lee Hanson allowed a pleasant change from a diet of salmon tours.

Donald Morden and the employees of Bioproducts, Inc. provided a tour of their production facilities.

And lastly, for the warm reception and enjoyable time at the Newport Science Center aquaculture facilities we thank Ed Kolbe and his staff.

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Thanks also go to Jeanne Larson for her diligence in proofreading the manuscript and to Carole Romberg for publication layout.

Contents

To Dr. Tadayoshi Ichihara

This volume is dedicated to Dr. Ichihara, whose death on December 16, 1981 shocked and saddened the participants of the North Pacific Aquaculture Symposium. He was a respected scientist, a prolific writer, and will be missed by all his close friends within the scientific communities of Canada, the Soviet Union, the United States, and Japan.

Throughout his professional career, Dr. Ichihara was involved with international problems and research on fish and mammals within the waters around Japan and the North Pacific. His areas of interest included: research on whales and fur seals; tagging and tracking chinook salmon; and particularly the use of biotelemetry to study the behavior of fish such as kokanee, yellowtail, sea bream, chum salmon, and skipjack.

Dr. Ichihara's first appointment was to the scientific staff of the Whales Research Institute. After receiving his doctorate from the University of Tokyo, he joined the Tokai Regional Fisheries Laboratory and the Far Seas Fisheries Laboratory, and at his death, was professor of behavioral ecology of marine mammals on the Tokai University faculty of marine science and technology.

Dr. Ichihara was instrumental in formulating the four nation aquaculture symposia, including the North Pacific Aquaculture Symposium. The next in the series, now scheduled for 1983, will be hosted by Tokai University. Dr. Ichihara had been named to head its Japanese delegation.

It is an honor to dedicate these proceedings to the memory of Dr. Ichihara for his record as a scientist and his role in the formation of these symposia.

Preface

During the Eighth Soviet-Japanese Symposium on Aquaculture and Raising the Biological Productivity of the World Ocean (Kiev, September 1979), scientists from Canada, Japan, U.S.S.R. and the U.S.A. agreed to the concept of a four nation scientific exchange in the field of aquaculture. The University of Alaska in cooperation with Oregon State University and the University of Washington hosted this scientific exchange.

Because of the importance of aquaculture to the Pacific Northwest, the planners of the Symposium decided to hold the meeting in both Alaska and Oregon. It was hoped this would allow more of the scientists from each region to participate in the scientific exchange and to interact with the scientists of the foreign delegations.

Introduction

The North Pacific Aquaculture Symposium was held 18 to 29 August 1980 beginning in Anchorage, Alaska, proceeding through Washington and Oregon and concluding in Seattle, Washington.

The official delegations were composed of the following members:

U.S.S.R.

Galena Aniskina
Dr. V. L. Kostarev (head)
Dr. Felix N. Roukhlov
Dr. V. P. Tumanov

Japan

Dr. Tadayoshi Ichihara
Dr. Mitsuo Iwashita (head)
Dr. Munehico Iwata
Dr. Tadashi Kubota
Dr. Katsuyoshi Mori
Dr. Sigeru Matoda
Dr. Yuji Nishihama
Nobuo Tokumatsu
Dr. Takimo Tsujita
Dr. Yasuo Yone

Canada

Dr. Don Alderdice
Dr. Craig Clarke
Terry Gjernes
Dr. Michael C. Healey
Ted Perry
Dr. F. K. Sandercock (head)

U.S.A.

Clinton E. Atkinson (head)
Dr. Willard E. Barber
Milo Bell
Dr. Walton W. Dickhoff
Dr. Lauren R. Donaldson
Philip Downey
Kurt L. Fresh
Dr. John L. Fryer
John B. Glude
Dr. Roger S. Grischkowsky
Dr. John H. Helle
Dr. William K. Hershberger
Dr. Bernard M. Kepshire
J. P. Koenings
Dr. Kenneth Leon
Dr. Tsuneo Nishiyama
Dr. Donald E. Rogers
Dr. Charles A. Simenstad
Stacia A. Sower
Dr. Gary A. Wedemeyer
Dr. John W. Zahradnik

The technical sessions were structured to include areas of interest to all four participating countries. In addition to the scientific presentations, a number of field trips were made to personally acquaint the foreign delegations with the work being carried out in various hatcheries. Since the symposium traveled from state to state, it also allowed time to see some spectacular scenery and learn a little about our way of life.

Members of the Symposium Committee were:

Symposium Chairman	Donald H. Rosenberg
Program Chairman	Richard A. Nevé
Facilities & Arrangements	Brenda R. Melteff
Field Trips	Raymond S. Hadley
Steering Committee Chairman	Clinton E. Atkinson

The overall structure of the symposium was developed by a steering committee whose members were:

Dr. Vera Alexander, Director
Institute of Marine Science
University of Alaska

Dr. Donald F. Amend, Director of Research
Tavolek Inc.

Mr. Ed Cummings
Oregon Department of Fish and Wildlife

Dr. William Heard, Director
Aquaculture Program
National Marine Fisheries Service

Mr. Armin Koernig, President
Prince William Sound Aquaculture Corporation

Dr. William McNeil, General Manager
Oregon Aqua-Foods, Inc.

Mr. Charles Meacham, Director
International Fisheries and External Affairs
Office of the Governor

Dr. Roy Nakatani
Washington Sea Grant Program
University of Washington

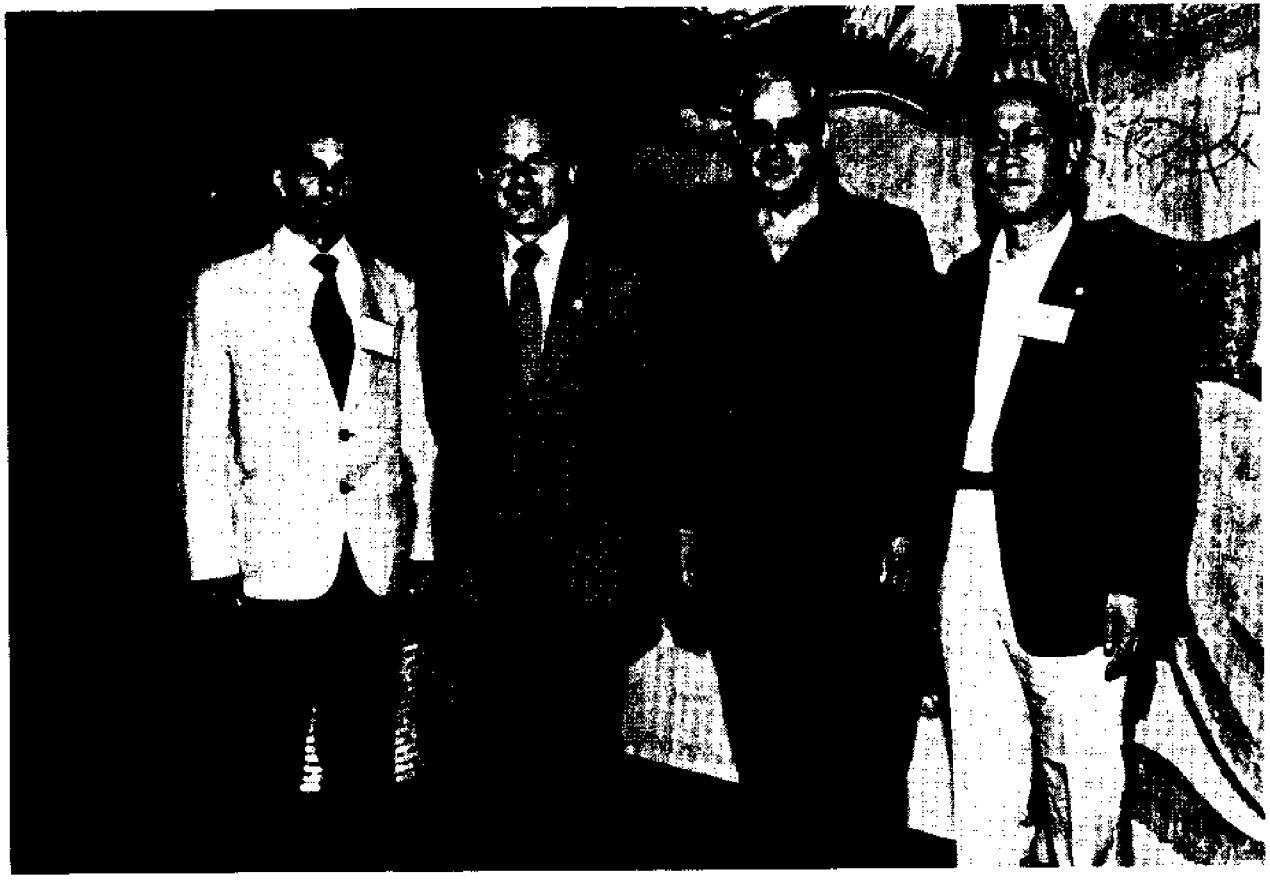
Mr. Robert S. Roys, Director
Fisheries Rehabilitation, Enhancement and
Development Division
Alaska Department of Fish and Game

Mr. Robert R. Simpson
National Marine Fisheries Service

Mr. William Q. Wick, Director
Sea Grant College Program
Oregon State University

Mr. Einar Wold, Director
Columbia River Fishery Development Program

Dr. Peter Bergman, Assistant Director
Washington State Department of Fisheries



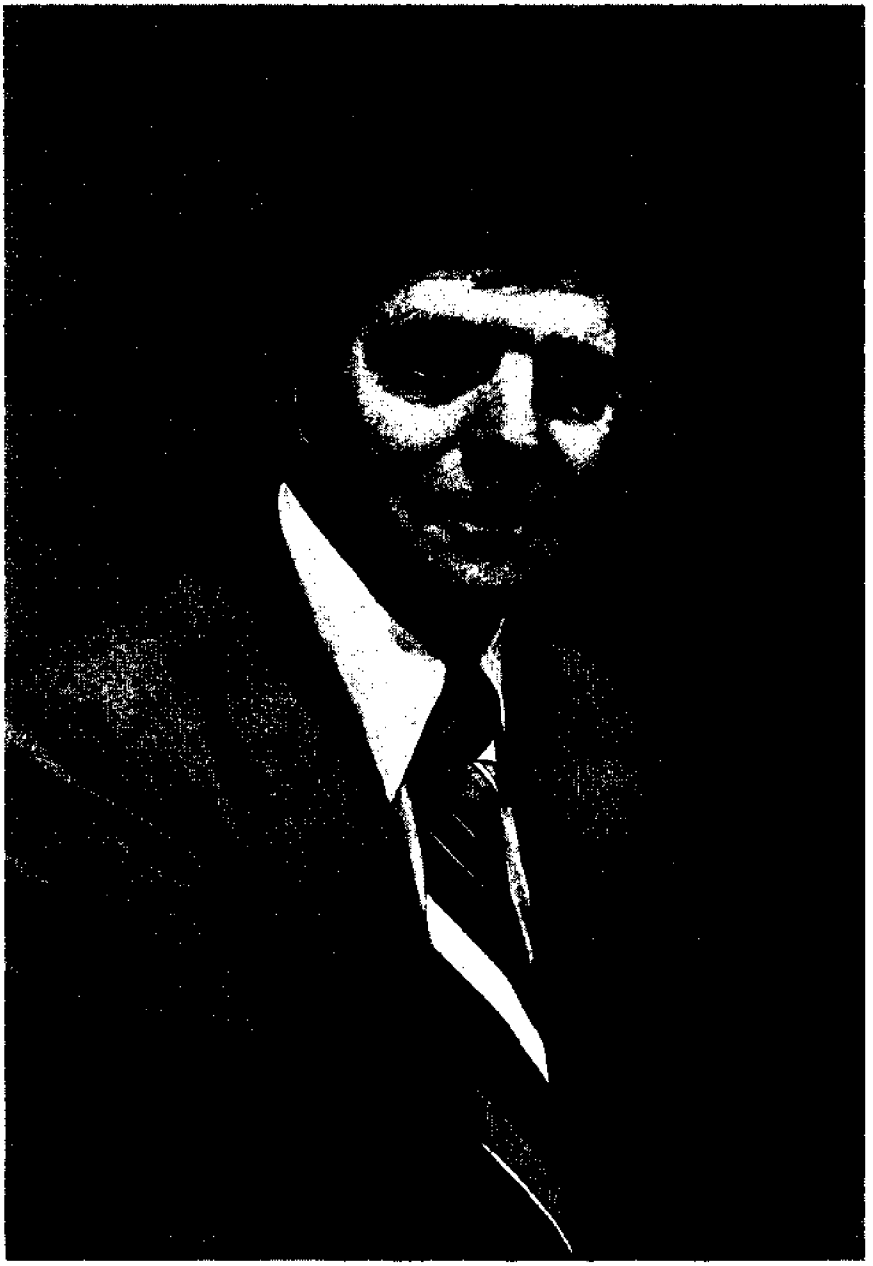
Heads of Delegations

Delegation from the Soviet Union

Delegation from Japan

Delegation from Canada

Delegation from the United States



Welcome

Jay Barton
President
University of Alaska

I am most pleased on behalf of the University of Alaska to welcome you to the North Pacific Aquaculture Symposium. We are honored by the participation of scientific delegations from Japan, Russia and Canada.

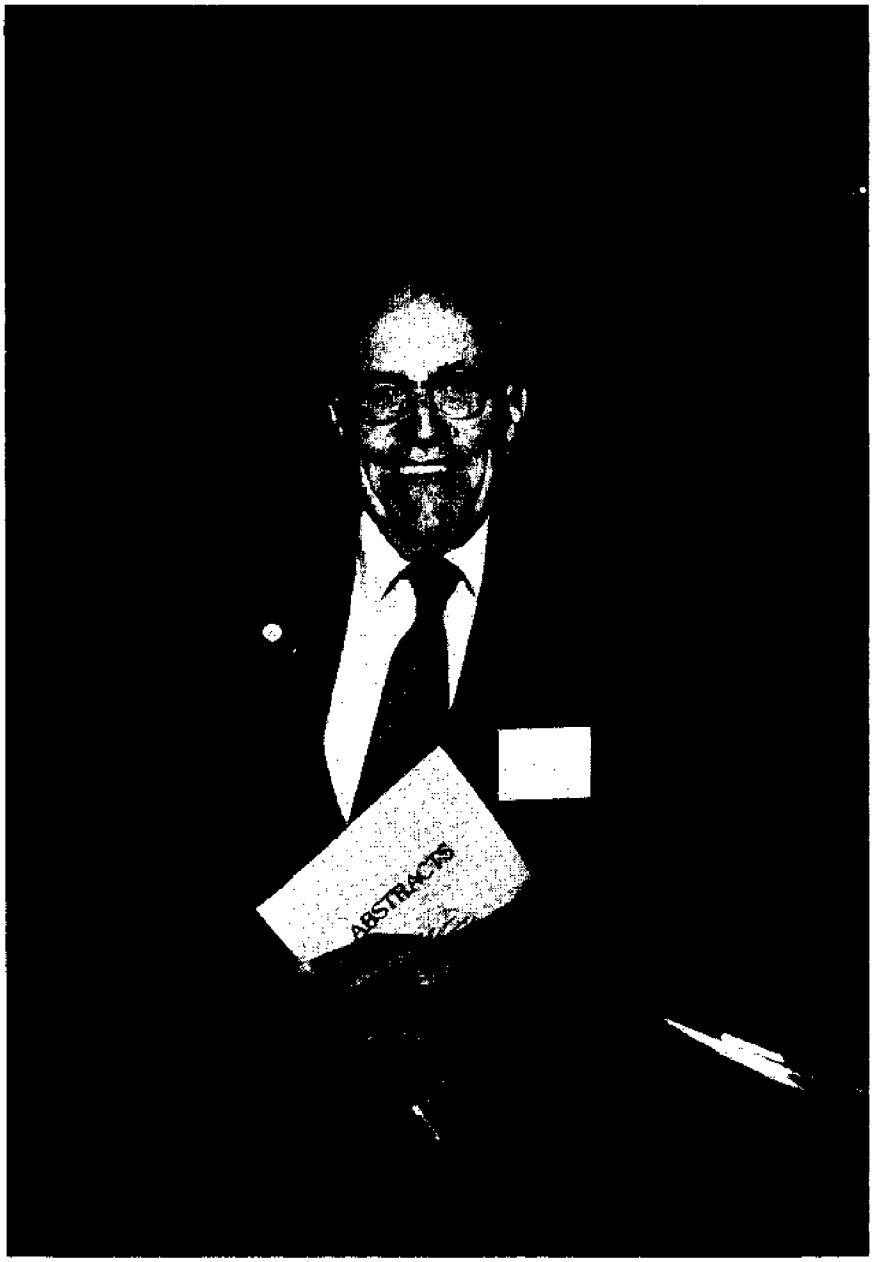
The problems of aquaculture with which you will all be dealing during the coming week are of greatest importance, not just to Alaska but to the world. The needs of the people of the world for high quality protein continue to increase. Our responsibilities as scientists, academicians, fishermen and citizens to protect our marine food resources by aquaculture and by management increase at a parallel rate. We are especially optimistic that we can move beyond the hunting and foraging and the ranching stage to a true intensive aquaculture. Farming the sea is possible but still in its infancy.

The University of Alaska is committed to a major development effort in cooperation with the fishing industry, with the State of Alaska and with the Federal Government to do just that. We hope that our fisheries program can provide the research studies, the education and development of people, the development of technology and of management strategies and especially with our marine advisory service, to bring all of this knowledge to the industry itself.

It is important to note that one of Alaska's great pioneers in fisheries understood clearly the complex relationships of biology, oceanography, processing technology, marketing development, management and entrepreneurship, etc., all necessary to make the whole system go. He understood how in the true land-grant or sea-grant concept a university puts together research and instruction to the service of the people. The pioneer of whom I am speaking is Lowell Wakefield who was responsible for the development of the king crab industry in Alaska. We might note that during his lifetime Lowell was named a Distinguished Associate in the University of Alaska Sea Grant Program. Lowell's wife participated in many of his activities. She is with us today. I'd like to welcome Mrs. Frankie Wakefield.

You will note that the symposium is sponsored by the Alaska Sea Grant Program, the Pacific Sea Grant Advisory Program, the Washington Sea Grant College Program and the Oregon Sea Grant College Program. Professor Donaldson has informed us that on his motion the Secretary's Advisory Committee has voted to elevate the University of Alaska's program to College status.

On behalf of the University of Alaska and the Alaska Sea Grant College Program I wish you all success in your deliberations and in your investigations. Good luck.


ABSTRACTS

Proc. No. Pac. Aquaculture Symp.,
Aug. 1980, Anchorage, Alaska

Keynote Address
The Past, Present and Future of Aquaculture

Lauren R. Donaldson

(Professor Emeritus of Fisheries, College of Fisheries, University of Washington, Seattle, Washington)

It is indeed a great personal pleasure for me to address the opening session of the North Pacific Aquaculture Symposium with scientists from Canada, Japan, U.S.S.R. and the United States in attendance. Those of us who were privileged to have attended the very productive International Meeting on Pacific Salmon held in South Sakhalinsk, U.S.S.R. 1 to 18 October 1978, are very happy to again be with our colleagues from overseas and look forward to a continuation of the information exchange they so effectively initiated.

This international gathering of aquaculturists, here in the State of Alaska and then moving on to Washington and Oregon, represents the culmination of the plans, efforts, and hopes of many dedicated people and the cooperative input of the University of Alaska, Oregon State University, and the University of Washington.

This Symposium, supported by the National Sea Grant Programs of the three sponsoring universities, marks another milestone in the emerging world interest in aquaculture. In 1866, the U.S. Congress passed the Land Grant Act that has supported, through Land Grant Colleges, much of the agricultural development in education and research in the United States. This program has been so successful with agriculture in the U.S. that we are the best fed people in the world.

Two hundred years ago almost 100 percent of the people in the United States were engaged in farming. Even the small percentage of the population not completely involved in food production and associated activities were partially self-sufficient, for they had a big garden, some chickens, and often a family cow. By 1935, the number of farmers in the total population had fallen to 25.3 percent of the total. By 1975, 40 years later, a time span many of us in this room have direct knowledge of, the United States farm population had fallen to 4.1 percent of the total population. In other words, 95.9 percent of the people are dependent upon these few for the food we eat.

One hundred years after the Land Grant Act, the U.S. Congress passed the Sea Grant Act. The original Sea Grant College Act of 1966 was modified in 1976 with enabling legislation: "to increase the understanding, assessment, development, utilization and conservation of the nation's ocean and coastal resources by providing assistance to promote a strong educational base, responsive research and training activities and broad and prompt dissemination of knowledge and techniques."

Support for aquaculture research is one of the mandates of the Sea Grant College Program. It is hoped that with the passage of time and a broad international effort, the farming of the sea will become a reality.

A moment ago I used the term "farmers." I am well aware there are differences in the names applied to increasing our supplies of food from the sea. We do, however, have one thing in common—we farm fish in the broadest sense, and ***we are here because we do not have enough fish.*** The role of the fisherman to harvest from the "wild" stocks no longer provides for the needs of the expanding population.

When I entered the field of fisheries as a profession over 50 years ago, we were lulled into thinking the ocean was vast and held inexhaustible resources. We all know how misleading that concept turned out to be. A few of us, interested in increasing our supply of some of the more valuable fishes by "farming" them, were often held up for ridicule as being dreamers relegated to the outer fringes of public acceptance.

It is a big thrill now to be able to review some of the highlights of the past 50 years and to point with pride to some of the accomplishments. We fish farmers have at long last evolved into fitting Webster's definition—to farm—"to engage in the business of raising crops or livestock." The only difference is that our "field" is the North Pacific Ocean. I'm sure we will have many interesting experiences during the next few years as members of this Symposium "show and tell" about their programs.

Before we get completely enmeshed in the great diversity of subjects listed for the present Symposium, it might be well to again repeat the words of a very wise man who said, "To ignore history is to be condemned to repeat it."

In his delightful, informative book, *Aquaculture in Southeast Asia*, Dr. Shao-Wen Ling describes the history of aquaculture from its early beginnings, some 4,000 years ago to the present. He makes the summary observation that traditional practices had been sufficient to satisfy the needs of a simple, rural-agrarian mode of life; but in this day of technology they are not good enough. Change is necessary, but how much, and how fast?

To many of us, commercial oyster culture has always been the epitome in marine aquaculture. Tidelands could be purchased or leased and managed just as the land farm, and the food of the oysters is available in the big pasture. Historically, seed for the oyster farms was dependent upon natural spawn that settled on the beds or was purchased from dealers who collected the spat on shells in areas of abundant spat production. Now, thanks to the pioneering work of Dr. Loosanoff and others, it is possible to spawn oysters from selected stock, rear the larva and collect them on shells for sale to the oyster farm.

Further advances in the technique of oyster seed production now reduce the cost and labor of transport and spreading the shell on the beds. The farmer can now buy a ball of spat of select stock, carry it to his farm, and place it in warm seawater, where the spat will set on crushed shells. After a few days growth the shells can be placed on trays or beds to grow. With the site for the oyster farm available and quality seed, the oyster farmer now needs only a quick way to shuck his crop to be in business.

The culture of the Pacific salmon started in 1870 on the McCloud River in the State of California. *The Manual of Fish Culture*, first published in 1897 and revised in 1900, describes in detail how eggs used for propagation were obtained from salmon as they migrated upstream to the natural spawning grounds. For the Baird Station on the McCloud River the eggs were collected from fish caught in large numbers in drag seines. The chinook salmon eggs from the Baird Station were shipped to many states and foreign countries and most transfers failed; one notable exception was New Zealand where runs were established that have flourished for a century.

At the Clackamas Station in Oregon, a rack was built across the river to stop the fish so they could be netted by fishermen standing on the rack at night.

In 1891, the Washington State Legislature appropriated $15,000 for a salmon hatchery on the Kalama River, with an egg taking station on the Chinook River. Again, eggs were collected by robbing the natural spawning grounds.

In 1907, the Green River hatchery was built on Soos Creek and chinook salmon eggs were

brought from the Kalama to start the runs that eventually produced seed for supplying other hatcheries subsequently built in the Puget Sound area.

From these four "donor" stocks many of the chinook brood stocks around the world evolved. Not only were migration paths changed but spawning times and outmigration patterns were changed by natural and purposeful selection.

Here in the State of Alaska aquaculture has had a long history with varied experiences. Starting in the early part of this century a number of sockeye salmon hatcheries were built that collected millions of eggs and liberated millions of unfed fry that failed to survive; eventually all the early hatcheries were closed. In the past ten years there has been a revival of interest in aquaculture in Alaska by the state and private commercial aquaculture corporations. The concentration of effort is on the incubation and early rearing of pink salmon.

Following the spectacular development of the chum salmon hatchery programs in Japan and the U.S.S.R., the State of Washington has greatly expanded its chum salmon program. In 1978, a total of 45,788 adult chum salmon returned to the hatcheries and 90,686,167 eggs were collected for use in increasing the runs.

Coho salmon have always been considered the "bread and butter" fish of fish culture along the eastern Pacific. In 1978, a total of 202,492 adult coho returned to the hatcheries of the Washington State Department of Fisheries, and 134,875,396 eggs were harvested for incubation and subsequent rearing for release to the North Pacific pasture.

As salmonid aquaculture expands, a major problem is developing in providing fish food of adequate quality in amounts needed.

The composition and type of processing of hatchery diets used to feed salmonid fishes vary greatly, but all formulas depend on processed fish products for the primary protein sources.

Most of the diet formulas specify herring meal as the protein base. The world decline in herring populations has created a very real shortage of herring meal. The quality of the limited amounts of herring meal available has also dropped with the development of the herring roe industry that extracts the herring roe by salting and partial digestion of the mature herring. The resulting meal made from processed herring has a high salt content and limited content of poor quality oil.

Inferior quality meal and fish oil in the diets result in slower growth, poor quality smolts, and reduced contribution of hatchery fish to the fishery.

The present annual needs for hatchery food in Washington and Oregon of 37 million pounds require about 20 million pounds of fish meal-fish oil combination. To produce this quantity of high quality fish protein-oil base for the diets, 40,000 to 50,000 tons of low value fish and fish scrap would be needed.

In summary, the future of aquaculture looks bright. As evidenced by many of the papers to be presented at this Symposium, we are at long last beginning to consider aquaculture as an integrated system of carefully integrated parts—taking advantage of the progress made by earlier workers—and hopefully avoiding their mistakes, and developing a concept of "farming" the North Pacific.

Of utmost importance, however, are the people with patience, skill and energy to put the entire concept into practice—the real farmers, who must be free to operate with a minimum of legal-social restraints.

"The only place that success comes before work is in the dictionary."

Acknowledgement

This paper is Contribution No. 549, College of Fisheries, University of Washington, Seattle, Washington.

Section I
Marine Ecological Interaction

Proc. No. Pac. Aquaculture Symp.,
Aug. 1980, Anchorage, Alaska

Foraging Success as a Determinant of Estuarine and Nearshore Carrying Capacity of Juvenile Chum Salmon (*Oncorhynchus keta*) in Hood Canal, Washington

Charles A. Simenstad and Ernest O. Salo

(Fisheries Research Institute, University of Washington, Seattle, Washington)

Abstract

Migration rate of and habitat selection by outmigrating chum salmon (*Oncorhynchus keta*) juveniles in Hood Canal relate directly to availability of preferred prey organisms. Juveniles entering the Canal early in the outmigration period (February and March), especially naturally spawned chums less than 40 mm FL, encounter relatively meager prey resources in shallow sublittoral and neritic habitats. Rapid migration rates (7 to 14 km/day) during this period suggest a behavioral response to low prey availability might be immediate migration into regions with more prey. In spring, epibenthic and neritic zooplankton increase, and migration rates decline (3 to 5 km/day) as the juvenile salmon spend more time foraging in estuarine and nearshore habitats. During this period they start eating epibenthic harpacticoid copepods and gammarid amphipods in shallow sublittoral habitats, but upon growing to 45 to 55 mm FL, move into neritic habitats and start eating pelagic and nektonic organisms such as calanoid copepods, hyperiid amphipods, and larvaceans. This transition is theorized to be the result of prey resource depression and growth of the fish to the point that they can feed upon larger neritic organisms and avoid predators. Selectivity for large prey is quite apparent, however, in both epibenthic and neritic feeding modes and is attributed to 1) visual perception and active selection, 2) differential prey escape abilities, 3) functional morphology of the juvenile chum salmon, and 4) optimization of bioenergetic cost of foraging with nutritional value of prey.

We assume that size-dependent daily rations between 15 and 25 percent of the total body weight per day would be required to provide optimal foraging. Accordingly, the estimated carrying capacity of juvenile chums in Hood Canal for two week periods ranged from 0.03 to 0.65 fish/m^2 in shallow sublittoral habitats and from 0.01 to 0.07 fish/m^3 in neritic habitats. Assuming these interpretations are reasonable and the estimates valid, a number of alternative hatchery design and release strategies may be developed to reduce mortality of artificially propagated chum salmon.

Introduction

Only recently have factors believed to limit the production of juvenile salmonids in the Northeast Pacific included estuarine and nearshore marine influences—of which predation, by implication, may be the more deleterious factor affecting survival (Parker, 1968 and 1971; Wickett, 1958; Gilhousen, 1962; Peterman and Gatto, 1978). These early marine effects are extremely important to chum salmon (*Oncorhynchus keta*) which, because of their early emigration from freshwater at a small size, appear to require nearshore habitats and environmental conditions conducive to rapid growth (Parker, 1971; Healey, 1979).

The prevalent outmigration behavior of juvenile chum salmon involves rapid emigration from freshwater to shallow nearshore marine habitats (Neave, 1955; Bakkala, 1970) where epibenthic and neritic food resources are extensively fed upon during the initial period of residence (Kaczynski, et al., 1973; Feller and Kaczynski, 1975; Mason, 1974; Healey, 1979). While freshwater feeding prior to outmigration has been reported to be significant in British

Columbia (Sparrow, 1968; Mason, 1974) and the Soviet Union (Levanidov and Levanidova, 1957), initiation of feeding usually occurs in the marine environment in Puget Sound and many other regions. This period may, as such, constitute a "critical" life history stage for chum salmon in terms of feeding, growth, and escape from natural mortality forces such as predators and disease. In this context, the role of food limitation in estuarine and nearshore marine environs may be a major determinant of the production of chum salmon (Healey, 1979; Gallagher, 1980; Gunsolus, 1978).

Earlier research on the survival of chum salmon in northern Hood Canal, an inland fjord of Puget Sound (Figure 1), indicated that the marine survival of early (March) outmigrant juveniles tended to be lower and more variable than that of the late (April) outmigrating fish (Koski, 1975; Schroder, 1977). This suggested to us that juvenile chum salmon entering Hood Canal too early to take advantage of spring increases in epibenthic and neritic zooplankton may experience comparatively slower growth and higher mortality. We do not, however, have data illustrating either the mechanism of mortality nor how the mortality rates are distributed among the estuarine, nearshore marine and oceanic phases of the chum salmon's life history.

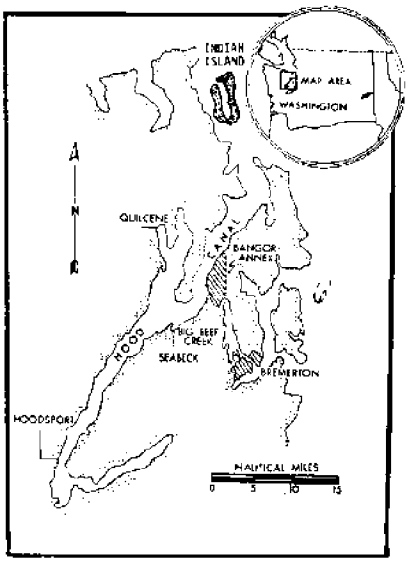


Figure 1. Location of Bangor Annex, Indian Island Annex, and fish hatcheries at Big Beef Creek, Quilcene and Hoodsport.

We hypothesized that limited prey resources and inadequate foraging success by early stock chums were responsible, at least in part, for the higher mortality rates. This suggested that, if the low densities of zooplankton were "limiting" under conditions in the early spring, the expanded zooplankton populations could, under heavy enough predation pressure, also be over-exploited by the relatively high densities of juvenile chum salmon which are released into Hood Canal from hatcheries in late spring. Various agencies, including state, federal, and tribal, are placing increasing priority upon the Hood Canal ecosystem as a principal chum salmon production and harvest region. In 1979, releases reached 50 million fish and they are expected to continue to increase (Figure 2). Thus, in terms of securing the success of these and other chum salmon enhancement programs, it appeared important to gather some basic knowledge of the functional relationships between estuarine and nearshore prey resources and the migration behavior, foraging success, growth, residence time, and mortality rates of outmigrating juvenile chum salmon.

We would like to describe our interpretation of some of these relationships as a result of three and one-half years of studies of chum salmon outmigrations in northern Hood Canal

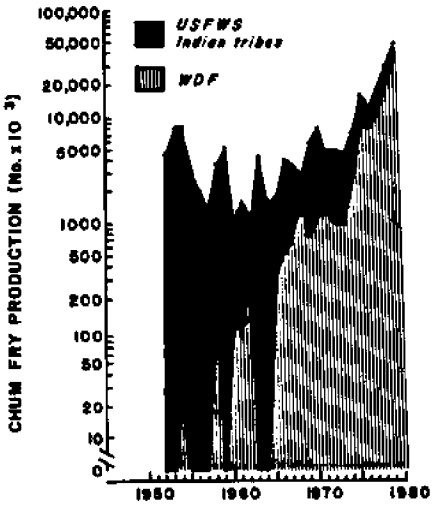


Figure 2. Numbers of chum salmon fry released annually into Hood Canal, Washington (1952-1980) from state, federal and Indian Hatcheries (from Cardwell, 1978).

by the University of Washington's Fisheries Research Institute (FRI, Simenstad, et al., in press). We would like to present what we believe is evidence for a direct relationship between juvenile chum migratory behavior and the abundance of preferred epibenthic and neritic prey populations. Our conclusion is that the scientific concept of "carrying capacity" may be an actual ecological phenomenon which, under some conditions, could limit the production of juvenile chum salmon in the Hood Canal ecosystem. We would also like to offer some arguments why and how this concept needs to be incorporated into future enhancement strategies for chum salmon in Puget Sound and perhaps other comparable regions.

Material and Methods

As a part of FRI's studies of chum and pink salmon outmigrations between 1975 and 1979 in northern Hood Canal, we quantitatively sampled the stomach contents of migrating salmon and the epibenthic and neritic zooplankton communities upon which they were feeding. Studies of the dynamics of the outmigration, migration rates through the Canal, mortality rates, and temporal and spatial distribution of juvenile salmon were also conducted concurrrently and provided important documentation of the migration behavior which we could relate to their foraging patterns.

Juvenile salmon were collected at representative locations on both sides of a portion of northern Hood Canal (Figure 3). Fish migrating through the shallow sublittoral habitats were sampled by beach seines and those in neritic waters were collected using a surface trawl or "townet." Epibenthic zooplankton were sampled using a suction pump system which drew water and associated plankters from within an enclosed sampling cylinder, through a flowmeter and into a filtration cylinder in which nested conical plankton nets were suspended (Figure 4). The finest mesh size of these plankton nets was 209 micrometers. Epibenthic zooplankton were sampled principally at three sites coincident with the most productive beach seine sampling sites. Surface neritic zooplankton were collected just offshore the three principal epibenthic sites using a standard 60 cm bongo net array equipped with 333 micrometer mesh nets. The contents of the stomachs preserved from representative subsamples of juvenile chums were examined and described in terms of fullness, stage of digestion, and taxonomic composition, frequency of occurrence, abundance, and biomass of prey organisms. Organisms were identified as specifically as their stage of digestion allowed and their life history stage reported if identifiable. Zooplankton collections were subsampled and the taxonomic composition, abundance and biomass determined.

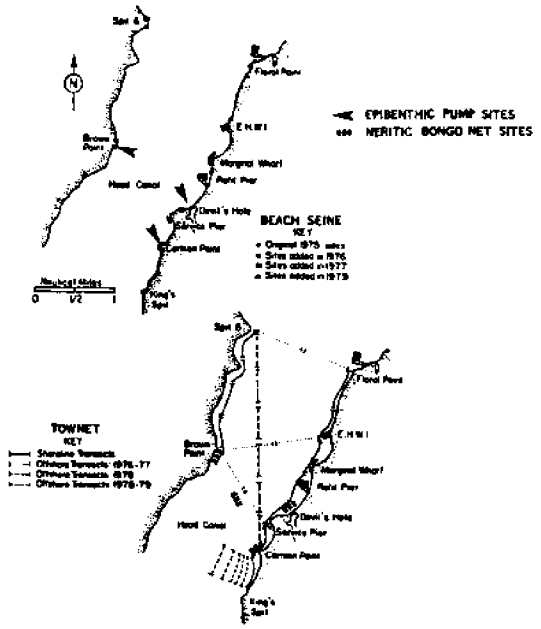


Figure 3. Beach seine sites, townet transects, and epibenthic and neritic zooplankton sampling sites in northern Hood Canal, Washington, from 1975 to 1979.

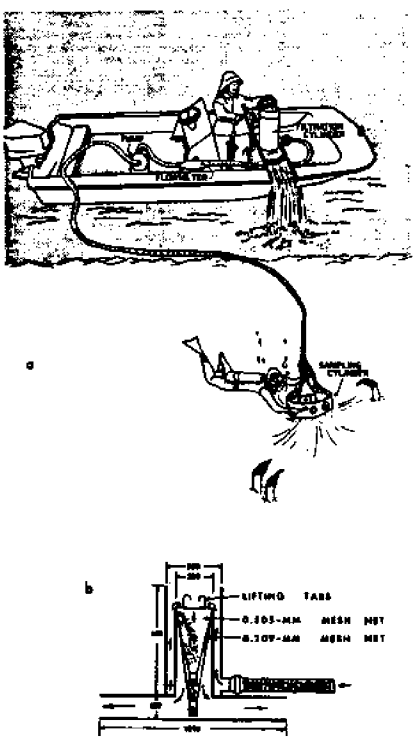


Figure 4. Components of epibenthic suction-pump sampling system as in use (a) and cross-sectional detail of filtration (b). All measurements in mm.

Results

When migrating through shallow sublittoral habitats, juvenile chum salmon fed principally upon epibenthic crustaceans, including primarily harpacticoid copepods and gammarid amphipods (Figure 5). The harpacticoid *Harpacticus* sp. and the gammarids *Calliopiella pratti* and *Ischyrocerus* sp. appeared to be the most common taxa. When feeding in neritic habitats, chums preyed upon calanoid copepods, hyperiid amphipods, euphausiids, and larvaceans (Figure 6). The common calanoids were *Calanus (pacificus x marshallae* complex), *Pseudocalanus* spp., and *Epilabidocera amphitrites;* hyperiid amphipods were primarily *Parathemisto pacifica;* and the euphausiids were unidentifiable larvae and juveniles while larvaceans were *Oikopleura* sp.

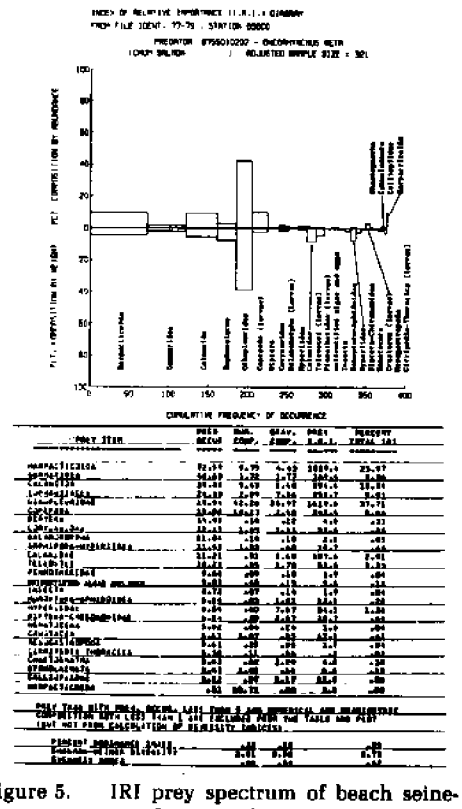


Figure 5. IRI prey spectrum of beach seine-caught juvenile chum salmon in Hood Canal, Washington, 1977-1979. Prey taxa have been pooled to the phylogenetic level of family or above.

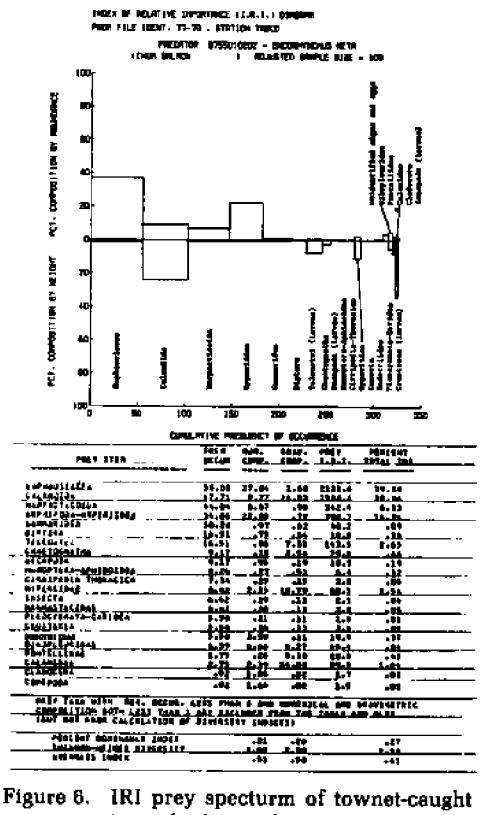


Figure 6. IRI prey specturm of townet-caught juvenile chum salmon in Hood Canal, Washington, 1977-1979. Prey taxa have been pooled to the phylogenetic level of family or above.

Whether the migrating salmon occupied shallow sublittoral habitats, feeding upon epibenthic organisms, or neritic habitats, feeding upon pelagic and nektonic organisms, appeared to depend upon the size of the fish and stage in the outmigration period. Immediately upon entry into Hood Canal chum between 30 and 40 mm FL (typically originating from naturally spawned populations or from the Big Beef spawning channel) fed upon epibenthos; as the fish grew or entered the Canal at a larger size (45 to 50 mm FL) (such as those released from hatchery facilities) they typically fed more upon neritic organisms. Temporal shifts in the prey composition of juvenile chums captured in shallow sublittoral habitats and those cap-

tured in neritic habitats illustrated that neritic fish had converted almost completely to neritic prey organisms by early May (Figure 7). Although there were several prominent pulses of neritic prey consumed by fish captured in shallow sublittoral habitats, their diet generally consisted of epibenthic organisms throughout the outmigration period until early July (Figure 8).

Based upon a diel feeding chronology study conducted in mid-May 1978, neritic chums appeared to feed in a relatively crepuscular manner, with peak feeding occurring at dusk and dawn (Figure 9). Prey composition also shifted over the diel period (Figure 10); large *Pseudocalanus* and *Calanus* and hyperiid amphipods appeared in the stomachs at dusk and dawn while smaller cyclopoid copepods tended to predominate in the stomach contents during daylight hours. We have no information that indicates prey composition of epibenthic-feeding chums varied as dramatically over the diel period.

Monitoring of the epibenthic zooplankton community indicated that chums were basically feeding upon the most common components of the epibenthos, as harpacticoid copepods and gammarid amphipods dominated the composition of the community. The majority of the fluctuations in density of epibenthic zooplankton was, in fact, a function of harpacticoid copepods (Figure 11). In all three years, after an initial increase in harpacticoid copepod density, a general decline in density coincided with peak densities of juvenile chum salmon in shallow sublittoral habitats (Figure 12). Continued monitoring of the epibenthic community in 1978, after the outmigration had ended and chums were no longer abundant, indicated harpacticoid density increased. This strongly suggests that the epibenthic-feeding fish could have depressed harpacticoid populations during the most intensive period of the outmigration. This could not be verified, however, without detailed taxonomic, population biology and ecological studies of the harpacticoid community, which was beyond the scope of the study.

Our collections of the neritic zooplankton community were much less extensive than those of the epibenthic community. Three collections spanning the 1979 outmigration were ex-

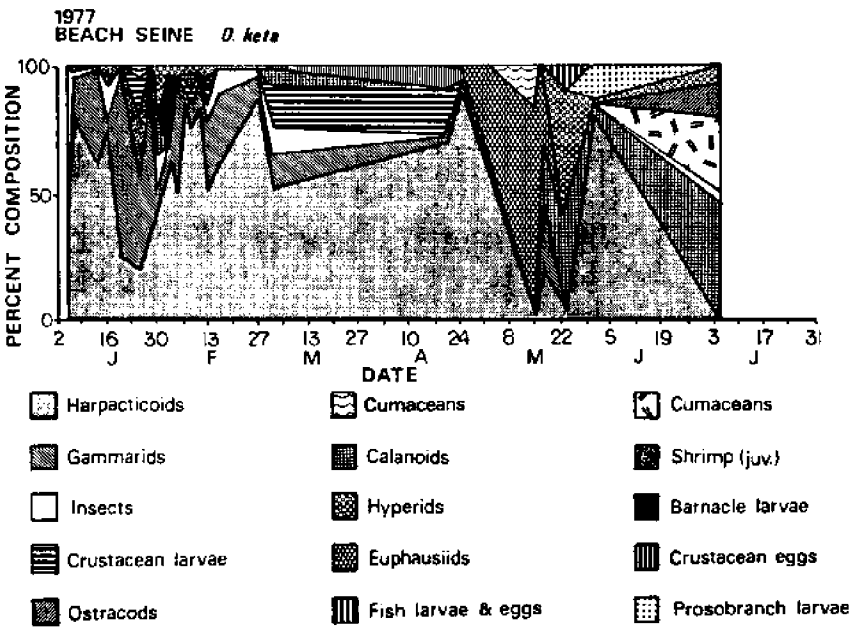


Figure 7. Prey composition (percent total Index of Relative Importance) of beach seine-caught juvenile chum salmon migrating through Hood Canal, Washington, 1977.

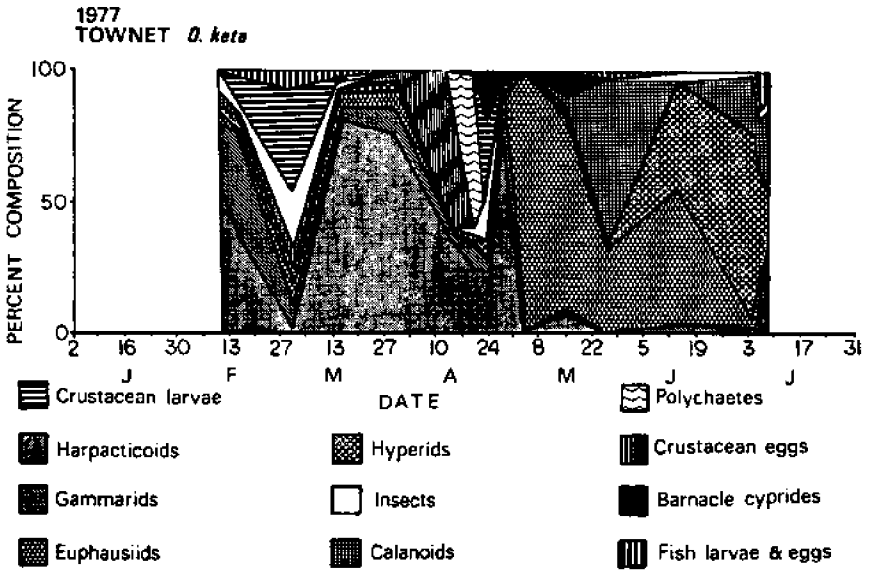


Figure 8. Prey composition (percent total Index of Relative Importance) of townet-caught juvenile chum salmon migrating through Hood Canal, Washington, 1977.

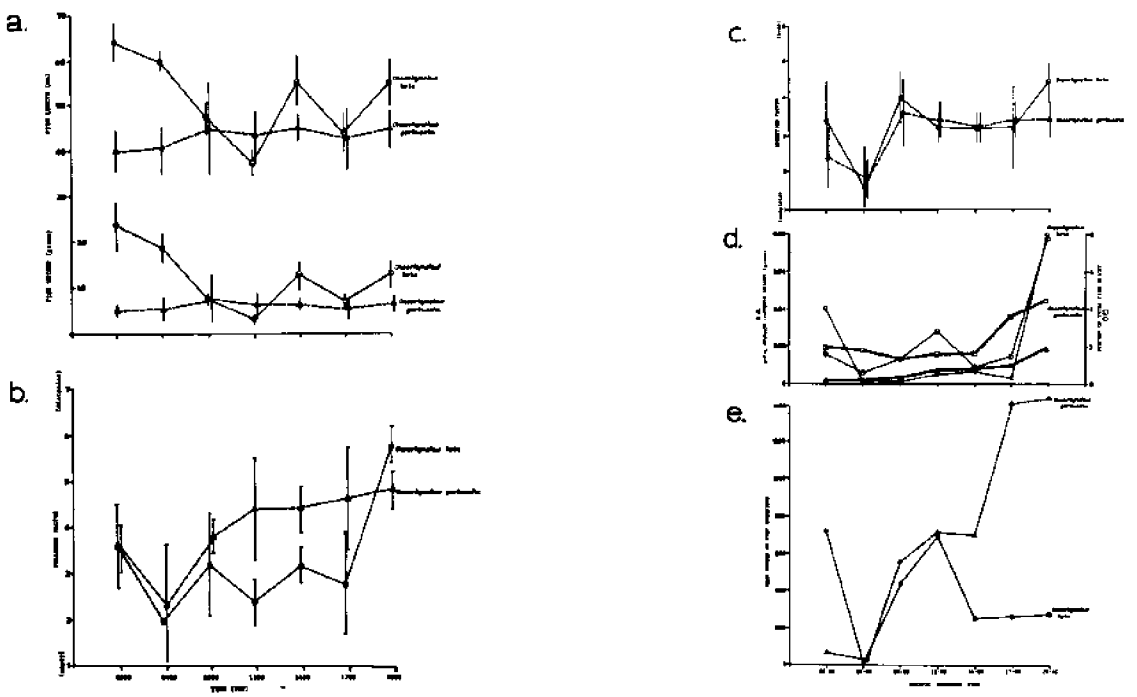


Figure 9. Fish length and weight (a), stomach fullness (b), stage of digestion of contents (c), stomach contents weight (d), and abundance (e) of prey organisms of juvenile chum and pink salmon during diel period, 15 May 1978, Carlson Point, Hood Canal, Washington.

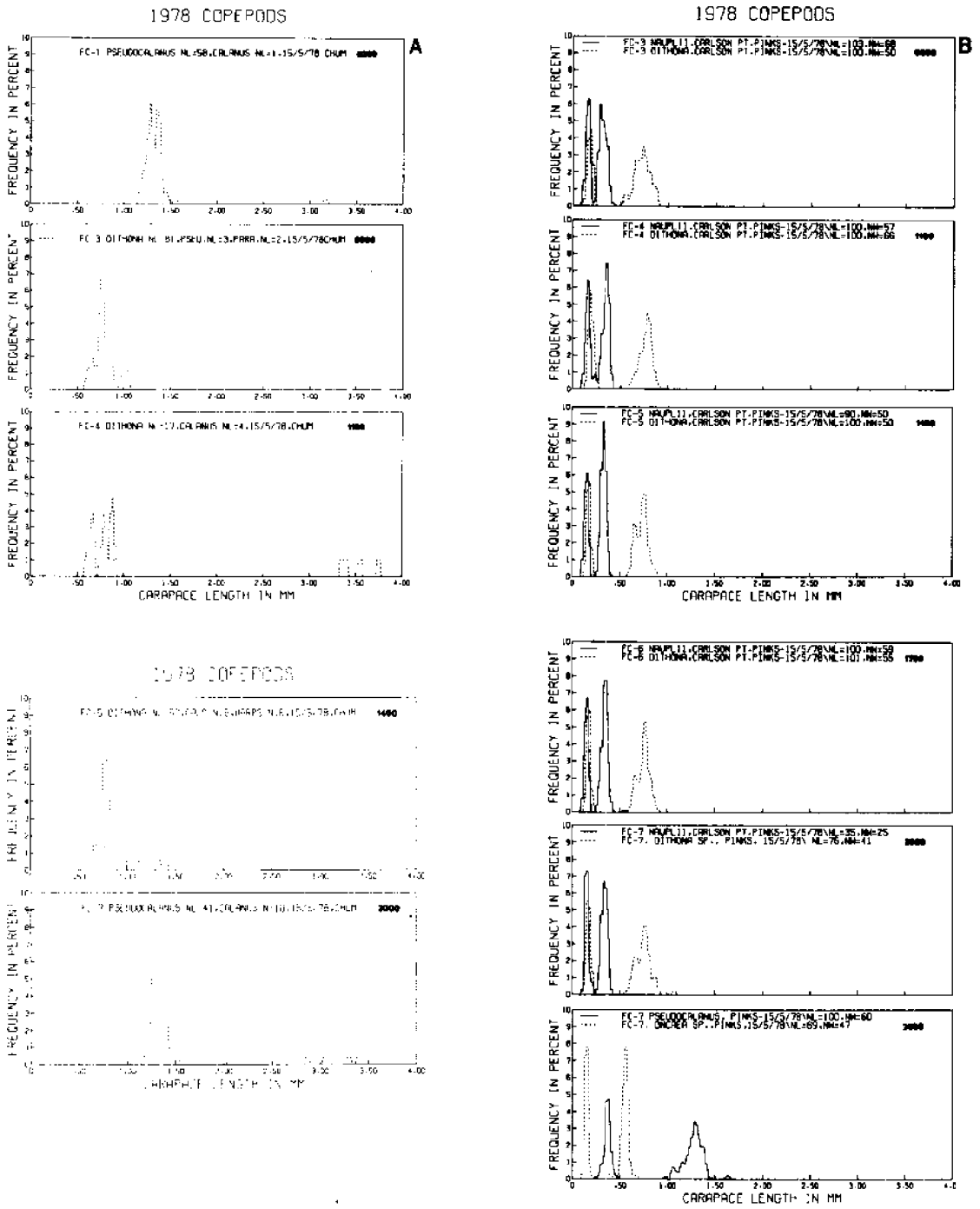


Figure 10. Prey size composition of organisms consumed by juvenile chum salmon (A) and pink salmon (B) over diel period, 15 May 1978, at Carlson Point, Hood Canal, Washington. Times are PST.

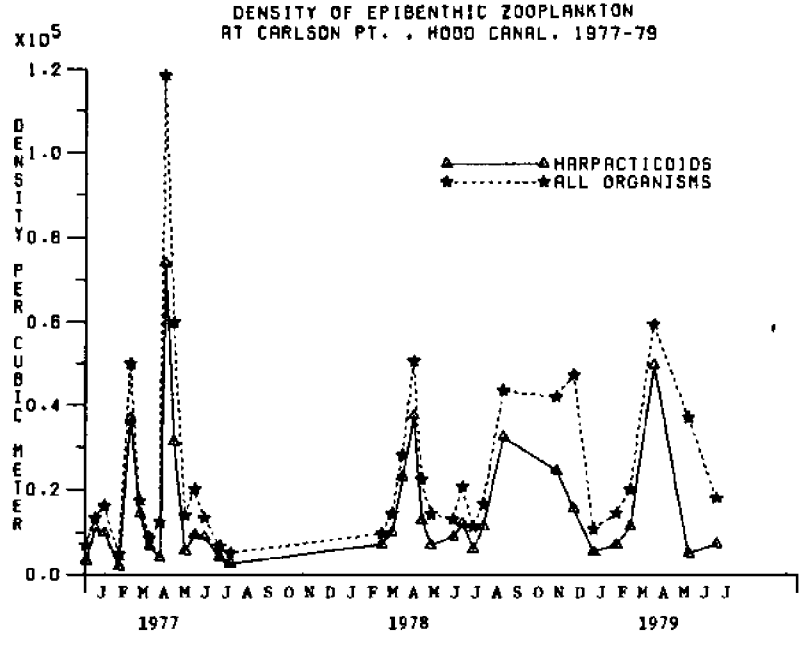


Figure 11. Mean total density (no./m³) of epibenthic zooplankton at Carlson Point, Hood Canal, Washington, 1977-1979.

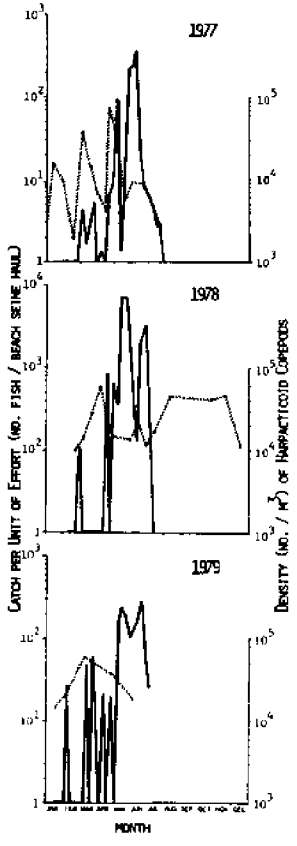


Figure 12. Density of epibenthic harpacticoid copepods (no./m³; ▬ ▬ ▬) and juvenile chum salmon (C.P.U.E. of 37-m beach seine, ▬▬▬) at Carlson Point, Hood Canal, Washington, 1977-1979.

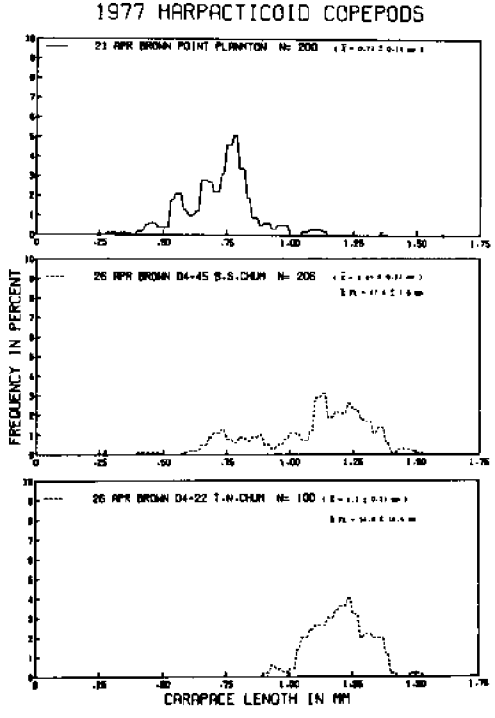


Figure 13. Harpacticoid copepod size (metasome length) distributions from epibenthic plankton community (a), and from stomach contents of outmigrating juvenile chum salmon caught in shallow sublittoral (b) and neritic (c) environments in Hood Canal, Washington, late April 1977.

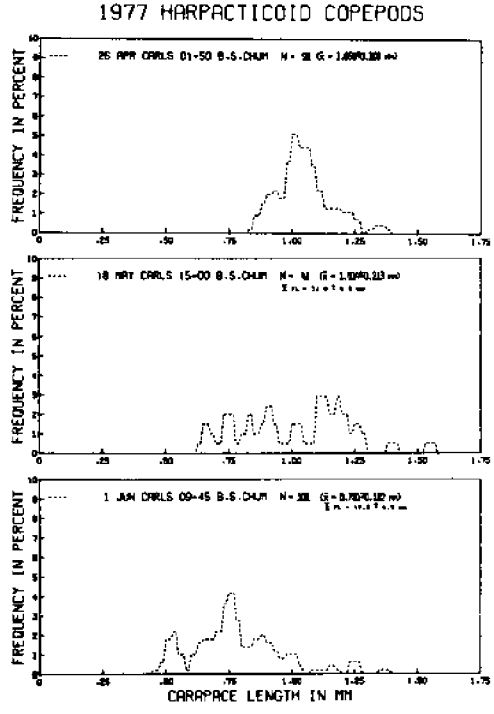


Figure 14. Harpacticoid copepod size (metasome length) distribution from stomach contents of outmigrating juvenile chum salmon caught in shallow sublittoral habitats in Hood Canal, Washington, late April-early June 1977.

amined. These indicated that the calanoid copepods *Paracalanus*, *Pseudocalanus*, and *Acartia* were the numerically dominant neritic zooplankton, with *Calanus* and *Tortanus* of secondary importance.

Based upon the comparisons of the concurrently collected stomach and zooplankton samples, juvenile chum salmon appeared to be highly size- and taxa-selective in their foraging in both shallow sublittoral and neritic habitats. Size-selection of harpacticoids and gammarids was readily apparent. The harpacticoids eaten by the chums often represent the upper 50 percent of the *in situ* size distribution and often were so large that they did not appear in the epibenthic pump samples (Figure 13), probably because they were so rare in the environment that our restricted sampling usually did not capture them. The mean sizes of the harpacticoids consumed by chum captured in neritic waters were also typically larger than those caught in shallow sublittoral habitats. There was also some evidence that intense size-selective predation upon the harpacticoid community was depressing the mean size distribution during the peak outmigration period (Figure 14) but, as with the associated decline in harpacticoid abundance, this cannot be verified without more complete knowledge of the biology of the specific components of the harpacticoid community. The epibenthic-feeding chums also appeared to select the largest of the available gammarid amphipods but the samples of stomachs containing numerous gammarids were too rare to make a definitive comparison.

Selective feeding behavior is even more evident in the neritic habitats (Figure 15). Ivlev electivity curves illustrated positive selectivity for larvaceans, chaetognaths, fish eggs, juvenile euphausiids and shrimp, and hyperiid amphipods, and negative selectivity for calanoid copepods in general, even though they were the principal prey. When species and size frequency distributions of calanoids in the stomachs of feeding chums and the environ-

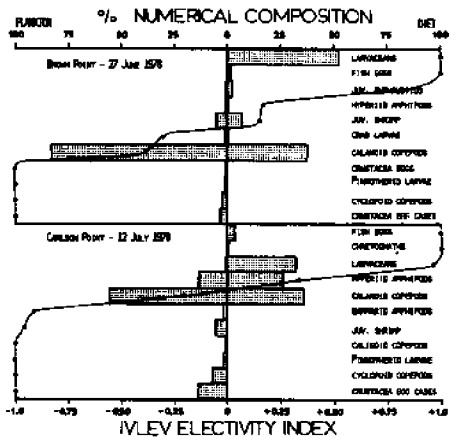


Figure 15. Ivlev electivity curves for juvenile chum salmon feeding in neritic waters adjacent to two sites in Hood Canal, Washington, June-July 1978. Plankton composition is derived from surface 60-cm bongo net tows and diet composition from chums captured by townet.

ment are compared, however, selectivity for large calanoid species is obvious (Figure 16), even though the two largest calanoids, *Calanus* sp. and *Epilabidocera amphitrites*, were extremely rare *in situ* compared to the abundant but smaller *Pseudocalanus*, *Paracalanus*, and *Centropages*, and the cyclopoid copepod, *Corycaeus*. Although not coordinated with these studies, oceanographic research in Hood Canal has provided evidence that *Calanus* are typically most abundant in the diffuse scattering layer which usually occurs deeper than 50 m during daylight in Hood Canal but which undergoes a diel migration into the surface waters during this time of year. This, in conjunction with the results of the feeding

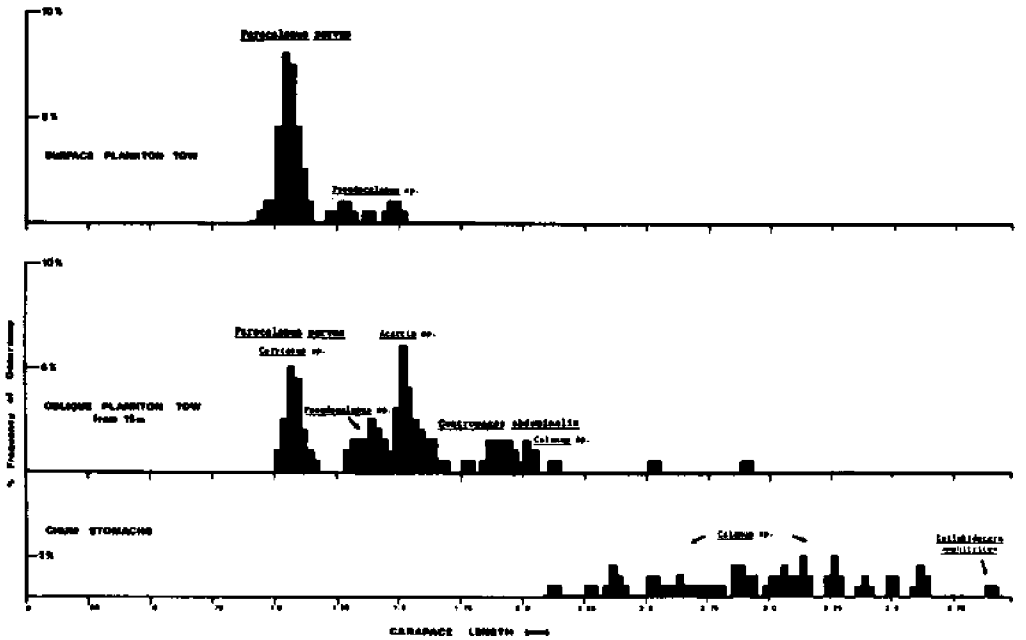


Figure 16. Size frequency of copepods from neritic zooplankton collections and from the stomachs of juvenile chum salmon caught by townet at Brown Point, Hood Canal, Washington, 27 June 1978.

chronology study, points to a highly selective feeding behavior by the juvenile chum salmon,wherein they optimize their caloric intake by feeding upon these large (although still relatively sparse) calanoids at dusk and dawn when their spatial distribution overlap; daytime feeding seems to be at a lower rate in proportion to the digestion rate of the stomach contents.

A variety of mechanisms could be responsible for this selection, including 1) differential visual perception, 2) more effective escape responses by the smaller prey organisms, 3) morphology of the chum's mouth or gillrakes, and 4) the bioenergetic budgeting of food capture time and effort versus the energy content of each prey organism. Other studies (LeBrasseur, 1969; LeBrasseur, et al., 1969; Koeller and Parsons, 1977) point to the bioenergetic argument, having shown that juvenile salmon have difficulty obtaining a required ration when consuming small prey even if the smaller prey were orders of magnitude more abundant than the larger prey. The principal consequence of this relationship would be increased foraging time or more rapid migration rates when optimally-sized prey are absent or scarce.

The results of the concurrent research on the migration behavior of the juvenile chum salmon lends support to the hypothesis that residence in shallow sublittoral and neritic habitats and speed of outmigration may be directly related to the availability and density of preferred prey organisms. Thus, a behavioral response to low prey availability would be immediate migration out of Hood Canal. In fact, migration rates in February and March were found to range between 8 and 14 km/day, decrease to 7 to 8 km/day in April and to be as slow as 3 km/day in June (Salo, et al., 1980). These migration rates result in total residence times in Hood Canal of hours to days early in the outmigration to weeks later toward the end of the outmigration, depending upon where the juvenile chum enter Hood Canal.

The choice of alternative feeding habitats, that is shallow sublittoral or neritic habitats, appears to have a number of constraints, at least in the case of juveniles less than 50 mm FL. Although they disperse into adjacent neritic waters at night to feed upon larger and perhaps more abundant prey, small juveniles may be forced back into shallow sublittoral habitats

during the day to avoid predators. Thus, even if there were depressed prey resources in the shallow sublittoral habitats, small juvenile chums may not have the behavioral option to escape into neritic habitats where prey may be abundant and of the preferred sizes.

Our results lend some support for the arguments for prey resource limitation upon the carrying capacity of juvenile chum salmon in Hood Canal, at least in terms of affecting the residence times and migration rates. If extended residence and growth in estuarine and nearshore marine habitats is desirable in order to reduce size-dependent mortality in the oceanic environment, then it would be worthwhile to adjust the densities, sizes, and temporal distribution of outmigrating chums to minimize the possibility that they encounter inadequate prey resources.

Accordingly, we have prepared a scenario in which we have made some initial, rough approximations of the densities of juvenile chums which could theoretically be supported in shallow sublittoral and neritic habitats at different periods of the outmigration. Our basic assumption is that at least two weeks residence time is desirable in both habitats; this would add approximately 2 cm to the total length of chum salmon under optimal growth conditions. Daily ration estimates used in these calculations varied from 25 percent of body weight per day for fish 35 to 40 mm FL to 15 percent of body weight per day for fish over 50 mm FL. The standing stock of zooplankton available for consumption has been adjusted by 1) the proportional representation of preferred (taxa and size) prey organisms, and 2) for production and recruitment by a daily production biomass ratio of 0.1. Using our 1978 zooplankton data, we found that the estimated biweekly carrying capacity for epibenthic-feeding fish declined-from an initial peak of 0.65 fish/m^2 in mid-February to 0.03 fish/m^2 in late June (Table 1). In actuality, these are estimates of "excess" or "surplus" carrying capacity since they were based upon estimates of epibenthic zooplankton already under predation by juvenile salmonids and a variety of other nearshore, epibenthic-feeding fishes and macroinvertebrates. Unfortunately, we have no data on the densities of either naturally-spawned chum salmon or the non-salmonids; the hatchery releases averaged about 1 million juveniles per week in April, when these fish would probably have initiated feeding upon epibenthic organisms (Salo, et al., 1980). At the most, these densities would have increased the total carrying capacity by approximately 0.5 to 1.0 fish/m^2.

Table 1. Estimated surplus carrying capacity of Hood Canal for epibenthic-feeding juvenile chum salmon during 1979 outmigration.

Date	Total prey wet biomass (mg/m^2)	Total biweekly prey production (mg/m^2)	Estimated biweekly carrying capacity ($fish/m^2$)	Total Hood carrying capacity (no. fish x 10^6)
Feb 21	819	1130	0.65	1.10
March 7	274	378	0.22	0.37
March 21	357	493	0.28	0.48
April 6	474	654	0.26	0.44
April 17	291	402	0.16	0.27
May 1	171	236	0.08	0.14
May 31	105	145	0.05	0.09
June 14	84	116	0.04	0.07
June 28	59	81	0.03	0.05

Our estimates of the neritic carrying capacity are more compromised in that they are based upon but a few daytime zooplankton collections rather than collections made during the crepuscular feeding period. Using the densities and estimated production of the five major preferred neritic prey organisms, the estimated biweekly carrying capacity was 0.01 fish/m^3 in early February and late April and 0.07 fish/m^3 in early June (Table 2). In the case of Hood Canal, these estimates imply that 1 to 2 million juvenile chums could be supported (biweekly)

Table 2. Estimated surplus carrying capacity of Hood Canal for neritic-feeding juvenile chum salmon over 1979 outmigration.

Date	*Calanus*	*Epilabidocera*	*Parathemisto*	*Oikopleura*	*Corycaeus*	Total neritic prey (mg/m^3)	Total biweekly prey production (mg/m^3)	Biweekly neritic carrying capacity ($fish/m^3$)	Total Hood Canal neritic carrying capacity (no. x 10^6)
Feb 6	2.5	—	1.8	2.2	0.6	1.71	4.10	0.010	1.0
Apr 27	9.6	—	—	118.1	7.0	1.73	4.15	0.010	1.0
Jun 5	8.2	1.4	0.6	51.4	3.3	12.43	29.83	0.071	7.1

in the Canal's shallow sublittoral habitats and 7 million chums in the neritic habitats over periods of peak zooplankton production.

These results suggest a number of strategies which could be advanced for optimizing the results of chum salmon hatchery release strategies, since the variables influencing the foraging and migrating behavior of the juvenile salmon—time, size, and density of release—are manipulatable by these enhancement facilities. The most opportune approach would be to monitor epibenthic and neritic zooplankton community structure and standing stock and adjust hatchery releases accordingly. There is the question of whether this is practical as it is, of course, an expensive undertaking. An alternative is to monitor the behavioral response of the outmigrating salmon to the *in situ* prey resources by determining their migration rates and residence times at various periods in the outmigration under different release strategies. Although this has usually involved extensive mark and recapture experiments, the recent developments in counting and measuring daily growth rings on otoliths suggest that this may be a simple, direct means of documenting estuarine growth and residence.

The effect of competition from naturally-spawned juvenile chums should also be considered in any enhancement effort, especially in the context of their ability to depress epibenthic zooplankton populations. This would suggest that releases from egg box incubators, which allow the volitional emigration of the juveniles into estuarine and marine waters, should not be designed around stocks naturally migrating at periods when epibenthic prey populations might be low and should be installed in regions with extensive shallow sublittoral habitat to support the epibenthic-feeding juveniles. At least the potential decreased survival rates which might result from such releases should be taken into consideration when assessing the cost-benefit ratios of egg box incubator systems.

Similarly, the manipulation of conventional hatchery releases should be considered in terms of the costs of the various options (e.g., number of days feeding before release, volitional or nocturnal releases) versus the estimated costs or gains in survival which could be predicted to occur as a function of the projected carrying capacity at that time. This may be especially important in the case of neritic-feeding juveniles which appear to be closely linked to unique prey resources such as *Calanus* copepods. The most direct way to implement such an evaluation of hatchery release strategies is, of course, to conduct full-scale production releases in the framework of an experimental design which includes the monitoring of the growth, behavior, and survival of outmigrating juvenile chums and the structure and standing stock of their prey resources.

All these proposals are predicted upon precise measures of estuarine and marine survival rates, something that is seldom documented and even less frequently incorporated into hatchery management strategies at this time. We are at a point in our science where, if we are to implement economically and biologically successful enhancement programs, we need to abandon the random, intuitive approaches in salmon propagation (relative to interactions with the marine environment) and conduct structured experiments with alternate release strategies based upon testable hypotheses concerning predicted survival rates. These will require a much more thorough knowledge of the behavior and ecology of juvenile salmon in estuarine and marine environments than we now have.

Acknowledgement

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Vertical Movement of Mature Chum Salmon Contributing to the Improvement of Set Net Structure on the Hokkaido Coast

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Introduction

With an increment of larvae of chum salmon released, the return of mature fish recently has been increasing in northern Japan. In order to effectively take eggs from mature salmon returning to home rivers from September through December on the Hokkaido coast, hatcheries with limited space need a constant supply of mature gonads. However, this has been often interrupted by the coastal net fisheries for salmon, since many trap nets are traditionally set in the coastal area. Predicting the amount of return for each year on scientific bases, several measures have been introduced for regulating competition between net fisheries and enhancement of salmon aquaculture. It is very hard to decrease the number of set nets throughout the season in order to obtain a constant run. A practical measure is to delay the opening date of net catch or to advance the closing date. It provides bimodal runs throughout the season, but net fisheries must take salmon efficiently during the peak of the season to compensate for the loss derived from the reduced catch period.

The structure of a set net is a factor in determining the catching efficiency. The slope of the ramp net is particularly important to conduct salmon from the playground into the trap. We have continued to examine the vertical movement of unrestrained mature salmon at sea through ultrasonic telemetry techniques and found the gradient of upward and downward movement of fish is smaller than the slopes of the ramp nets. This finding may coincide with the evidence that many fish escape from the playground of a set net, resulting in decreasing the efficiency of the catch. Our results will provide valuable information for improving the structure of the set net.

Telemetric Method for Behavioral Study of Salmon

Ultrasonic transmitters of 50 khz were made by the technical branch of our faculty in Shimizu. The transmitter in an epoxy capsule is 17 x 80 mm in size and its weight is 31g in air and 10g in water. The shape and basic structure of the transmitter are about the same as described by Ichihara, et al. (1975), but the circuit and the mechanical structure concerning a depth sensor are much improved. A very light silicon bulk semiconductor is effectively used as a depth sensor and the error of measurement by it is under 3 percent. Pressure directly changes the resistance of the sensor and subsequently modulates the pulse interval. The pulse is transmitted every two seconds on an average. The width of the pulse is determined as 20 ms from experience. Receiving apparatuses are the same as reported by Ichihara, et al. (1972).

In 1979, a behavioral study of chum salmon was carried out using 12 mature fish. Nine of them were captured in the Abashiri River which empties into the Okhotsk Sea and three were captured by set nets established along the coast. Each fish was released at sea with an additional two or three fish after they were transported from the capture site to the release point by a research vessel. Disc tags were fixed on dorsal sides of all fish. A transmitter was inserted into the stomach of a fish anesthetized with MS 222. It takes about one hour before the fish bearing the transmitter adapts to the burden of transmitter. The capture site in the Abashiri River is located 1.5 km up the mouth where the seawater comes up with the flood tide. It suggests that fish from the river may easily adapt to sea conditions.

The propagation range of the ultrasonic signal is under 2 km in the sea and the swimming depth is recorded at a range of 1 km in order not to frighten the fish. The position of the vessel was determined every 30 minutes by land bearing in the daytime and with a radar on cloudy days and at night. Thus, the position of fish is indicated by the position of the research vessel approaching the fish. Table 1 shows a list of chum salmon tracked by telemetric technique, periods of tracking and distances of movement, in the Okhotsk Sea, 1979.

Table 1. Chum salmon used for tracking and distance of movement on the Okhotsk coast, 1979.

No.	Date	Sex	Fork Length (cm)	Period of tracking (h. min)	Actual distance of movement (km) A	Straight distance of movement (km) B*	A/B
1	Sept. 27	F	66.0	4:03	6.3	1.7	3.70
2	Sept. 30	F	72.0	17:02	17.2	4.3	4.00
3	Oct. 4	M	68.0	10:00	8.7	7.9	1.10
4	Oct. 5	F	78.0	6:27	9.7	3.6	2.42
5	Oct. 7	M	61.0	5:22	12.2	4.2	2.90
6	Oct. 9	M	69.0	10:50	42.7	3.9	10.95
7	Oct. 11	M	70.0	8:00	28.6	6.1	4.69
8	Oct. 13	M	59.0	3:52	9.3	2.3	4.04
9	Oct. 14	F	68.0	15:48	40.5	21.0	1.93
10	Oct. 18	M	55.0	21:33	53.7	25.8	2.08
11	Oct. 23	M	66.0	14:30	78.7	26.2	3.00
12	Oct. 25	M	72.0	39:42	104.3	31.6	3.30

*The straight distance from the release to the last point of tracking.

Horizontal Movement of Fish

From 27 September through 25 October, a total of 12 fish were tracked and the swimming depth was recorded on an average of every two seconds. Periods of tracking ranged from 3 hours and 52 minutes to 39 hours and 42 minutes. Distance of horizontal movement differed from individual to individual, ranging from 6.3 km to 104.3 km. When the release point is connected with the last point of tracking by a straight line on a chart, the straight distance of movement is measured. The mean ratio of actual distance of movement against the straight distance is 2.97 (1.10—10.95), as shown in Table 1. This ratio suggests that the actual movement of chum salmon is more active than the movement estimated from tagging and subsequent recovery.

Swimming ground speeds are illustrated in Figure 1 throughout the duration of the experiments. The mean ground speed was 47.4 m/min (2.8 km/hour), while the maximum speed was 260 m/min (15.6 km/hour). This indicates that mature chum salmon on the coast move at a mean speed of 1.2 fork length per second.

The mean ground speed was 53.5 m/min in the daytime and it was 40.1 m/min at night. Chum salmon in the daytime may swim slightly faster than at night but there is a similar pattern in the frequency distribution of speed day and night as shown in Figure 2. Figure 3 shows percent frequency of ground speed for the flood and ebb tides. The mean ground

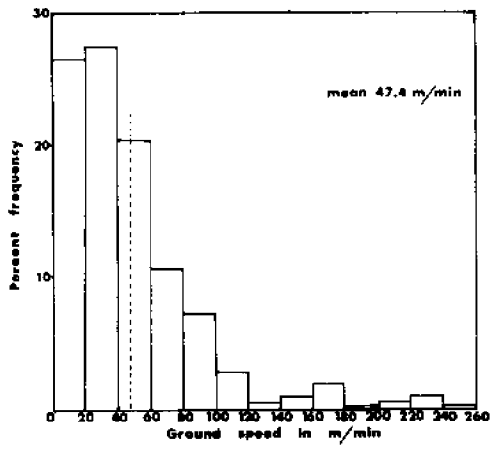

Figure 1. Frequency distribution of swimming ground speed of mature chum salmon on the Okhotsk coast, 1979.

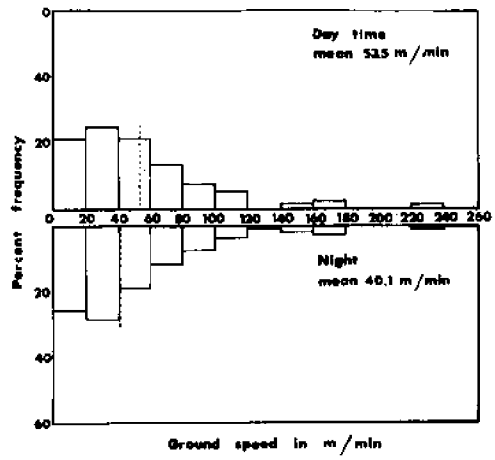

Figure 2. Swimming ground speeds of mature chum salmon in the daytime and night in the Okhotsk Sea, 1979.

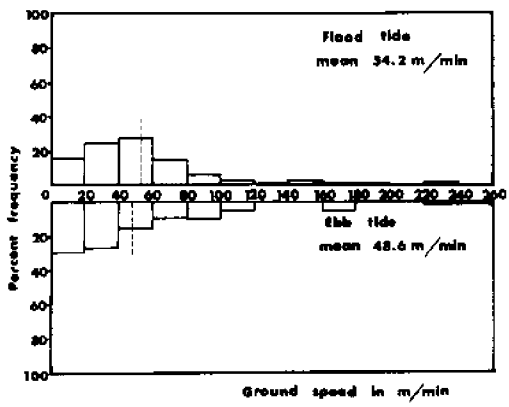

Figure 3. Swimming ground speeds of mature chum salmon in the flood and ebb tide on the Okhotsk coast, 1979.

speed in the flood tide was 54.2 m/min and 48.6 m/min in the ebb tide, indicating no significant difference between tides. For the purpose of this paper, the moving direction of each fish is not described here.

Characteristic Vertical Movement of Fish

Swimming Depth

Throughout experiments of 1979, the majority of salmon stayed in the 0 to 20 m layer in depth. Their stay in the 0 to 5 m layer occupied 43.9 percent of all telemetric records. The maximum depth fish reached was 100 m. The mean swimming depth was 13.7 m as shown in Figure 4. Percent frequency of swimming depth is compared between daytime and night in Figure 5. At night, fish stayed in the 0 to 5 m layer at a slightly higher frequency than in the daytime.

The mean swimming depth was 14.3 m in the daytime and 13.3 m at night, suggesting no remarkable difference in the swimming depth day and night. From Figure 6 it is seen that the mean swimming depth during ebb tide was 17.8 m, and 9.7 m during flood tide.

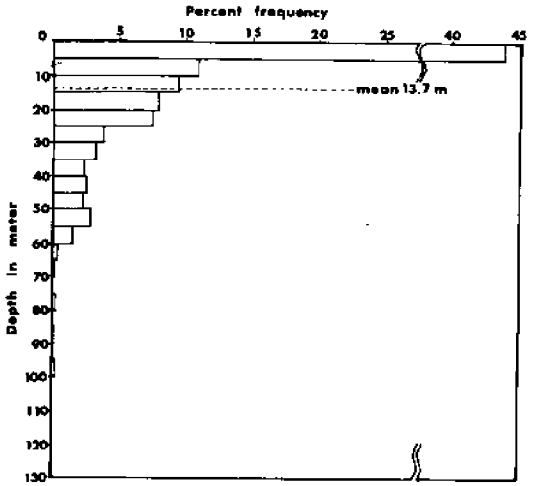

Figure 4. Frequency distribution of swimming depth of mature chum salmon on the Okhotsk coast, 1979.

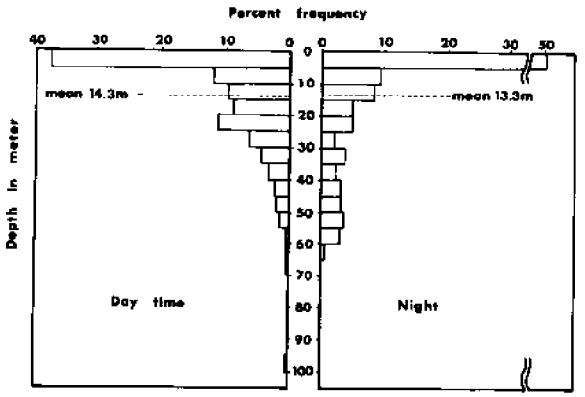

Figure 5. Swimming depth of mature chum in the daytime and at night on the Okhotsk coast, 1979.

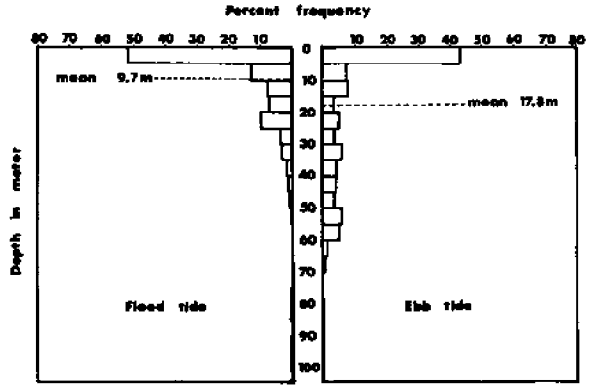

Figure 6. Swimming depth of mature chum salmon in the flood and ebb tide on the Okhotsk coast, 1979.

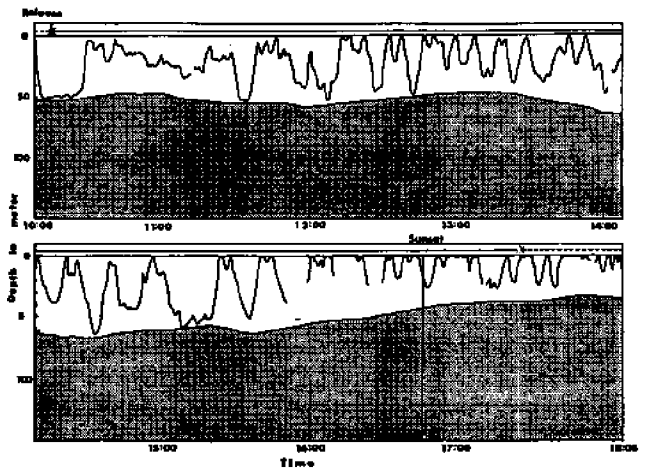

Figure 7. Vertical movement of mature chum salmon No. 7, during 8 hours after release on the Okhotsk coast, 1979. Flood tide: ————; Ebb tide: ————.

Upward and Downward Movement

Fish released from the vessel swam down rapidly, as they escaped from restraint. Then they again approached the surface of the sea and continued to move upward and downward. Records of Figure 7 indicate the characteristic vertical movements of fish No. 7 examined on the Okhotsk coast. Such vertical behavior appears usual for mature chum salmon approaching the Japanese coast. Ichihara, et al. (1975), found the same pattern in a fish examined off Etorohu Island, a part of the southern Kurile Islands. Ichihara and Nakamura (in press) observed this characteristic movement for fish examined in the Nemuro Strait, a northeastern part of Japan, in 1977. The Marine Ecology Research Institute in Japan (1979 and 1980) has examined an effect of the warm water flows from energy plants on the movement of mature chum salmon off the Fukushima Prefecture in northern Japan. Through a biotelemetric technique, it found similar characteristic behavior in vertical movements of fish.

Figure 8 indicates locations where the experiments described above were carried on. Although there are remarkable differences in the topography of sea bottoms and the environmental sea conditions among these locations, a similar vertical movement always appears. Figure 7 indicates that the fish continue to repeat the upward and downward

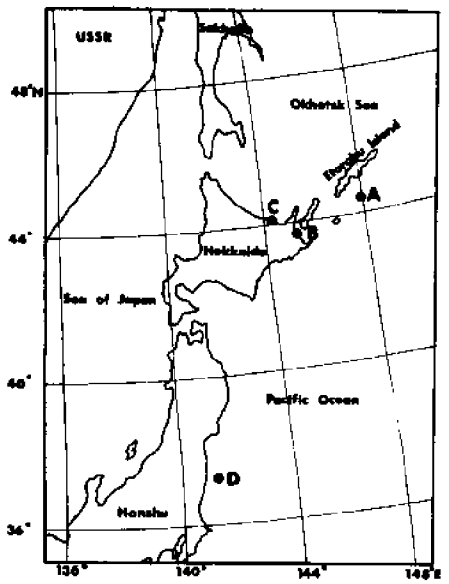

Figure 8. Locations where ultrasonic telemetric experiments have been carried out on the Japanese coast from 1974 through 1979.
A: off Etorohu Island
B: off Shibetsu in the Nemuro Strait
C: off Abashiri
D: off Fukushima Prefecture

movements in the daytime and at night, regardless of directions of tidal currents. In an interval of 10 to 30 minutes, fish come to the 0 to 5 m layer in depth, however, there is no accurate periodicity in the vertical movement. Such vertical movements are maintained even when the sea is very deep. Since salmon seldom penetrate thermoclines to go down, the maximum swimming depth of fish is regulated by the depths of thermoclines in the local waters (Ichihara, et al., 1975). There is a trend toward less vertical movement in shallow waters, hence the fish tend to stay in the subsurface layer.

Table 2 shows frequencies per hour when mature chum salmon come up to the 0 to 5 m layer in depth. From 1974 through 1979, the behavior of salmon was studied by the same telemetric system in four localities. The purpose of examination differs slightly from locality to locality, but we can reexamine the records of swimming depth. Salmon examined were captured by set nets established in the salt water or by another fishing method close to a

Table 2. Frequencies per hour in which mature chum salmon come up to the 0 ~ 5 meter layer in depth.

Location of experiment	Year	Month	Surface water temperature, °C	Location of fish captured	Number of fish used	Frequency
North Pacific (off Etorohu Island)	1974	Oct.	9.0 ~ 11.5	Sea	1	1.7
North Pacific (Nemuro Strait)	1977	Sept. ~ Oct.	17.0 ~ 18.5	Sea	6	3.0
Okhotsk Sea (off Abashiri)	1979	Sept. ~ Oct.	13.5 ~ 15.5	Sea	1	4.2
				River	6	4.2
North Pacific (off Fukushima Prefecture)	1978	Oct. ~ Nov.	15.0 ~ 19.0	Sea	10	5.1
				River	8	5.4
ditto	1979	Oct. ~ Nov.	17.0 ~ 21.0	Sea	8	7.6
				River	17	6.3

freshwater river mouth. The mean frequency per hour at which fish returned to the surface layer varied from 1.7 off Etorohu Island to 7.6 off the Fukushima Prefecture, indicating an increasing trend with the decrease of latitude. When surface water temperatures are taken into consideration, the frequency of upward movement seems to increase with an increment in water temperature. However, the frequency is 3.0 in the Nemuro Strait of 17.0 to 18.5°C for the surface water temperature while it is 5.1 off Fukushima Prefecture at a surface temperature of 15.0 to 19.0°C. In general, the maturity of gonads progresses further in the southern area than in the northern area, since salmon migrate from north to south for spawning. Hasler and Wisby (1951) and Hiyama, et al. (1967), emphasize that the olfactory sense plays an important role for homing of salmon in the coastal area. This characteristic vertical movement mentioned above probably relates to the olfactory function as suggested by Ichihara and Nakamura (in press).

Information on Improvement of Structure of Set Net

From the horizontal and vertical moving speed, gradients with which salmon descend and ascend in the sea are measured. Since free swimming salmon repeat the upward and downward movements in the coastal area as shown in Figure 7, we can estimate gradients from many available data. As there is no correlation between the horizontal and vertical moving speed, an average of ground speed can be a standard of horizontal vector for calculating gradients. Numbers of measurements as the basis for calculation are described in the last column of Table 3; 432 for the horizontal vector and 3,316 for the vertical vector. There are vertical movements of two types; one appears soon after fish are released from the vessel and the other occurs naturally in the free swimming state. In the former case, fish quickly descend to escape from the restraint and the descending gradient is larger than in the latter case.

Table 3. Gradient of nets conducting salmon into trap.

Type of set net	Slope of ramp net	Depth of sea (meter)				
		>18	18~36	36~54	54~72	72<
Trap set	Ascent	0.15~0.25 (22)	0.20~0.40 (16)	0.30~0.50 (15)	0.30~ (18)	0.30~ (12)
Bottom set	Descent	~0.15 (15)	0.20~0.35 (18)	0.30~0.40 (14)	0.30~ (7)	—
Midwater set	Ascent	—	0.12~0.15 (4)	0.15~0.30 (3)	0.15~0.30 (5)	0.20~ (4)
	Descent	—	0.12~0.20	0.20~0.35	0.20~0.40	0.35~

Parenthesis indicates number of nets examined.

The mean vertical moving speed in escapement is 18.9 cm/sec, while that in the natural state is 8.5 cm/sec. The mean vertical moving speed in the natural state off Etorohu Island was 7.6 cm/sec (Ichihara, et al., 1975). The escape movement does not continue long and the fish approached the subsurface layer within one hour after they were released. The mean ascending vertical moving speed was 9.00 cm/sec, slightly greater than the descending speed. These values are shown in Table 3. From these speeds, descending and ascending gradients are calculated for salmon. The mean gradient for escapement is 0.240, 2.2 times that for the natural descent. The gradient occasionally increases in the case of the natural descent, because fish often get away from the research and sailing vessels approaching them. Descending and ascending angles are calculated from these gradients and shown in the same table. On the basis of these values of angles, a schematic diagram can be drawn, as in Figure

9, which indicates a pattern of the vertical movement of mature chum salmon on the Japanese coast.

In autumn, many set nets of various types are used along the coast of northern Japan to take salmon during their spawning migration. The set net fishery is one of traditional fishing methods long recorded in history. Its basic characteristic is that fish move into the trap in a passive manner. Set nets recently used for salmon fishing are divided into three types in accordance with underwater positions of the trap as shown in Figure 10; trap set net, bottom set net and midwater set net. This figure illustrates a schematic diagram of the vertical section of salmon set nets. In general, the trap set net is a traditional one and suitable for shallow places of slow tidal currents while both the bottom and the midwater set nets are established in deep places of fast tidal currents. Fish conducted from the open waters into the playground are subsequently conducted into the trap through the ramp net. The specific structure of each type of set net differs. Some features of the ramp net appear in the lower side of the trap set net, in the upper side of the bottom set net, and in both the lower and upper sides of the midwater set net. These gradients seem to have an affect on conducting fish from the playground into the trap.

Data available on the slopes of ramp nets were collected from a total of 153 set nets established on the Hokkaido coast, comprising 83 trap set, 54 bottom set and 16 midwater set nets. Midwater set nets are not established in water under 18 m in depth. The ranges of gra-

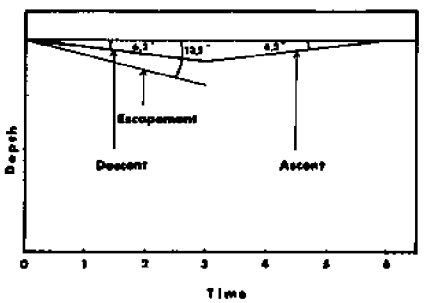

Figure 9. Schematic diagram indicating mean descending and ascending gradients of free swimming mature chum salmon.

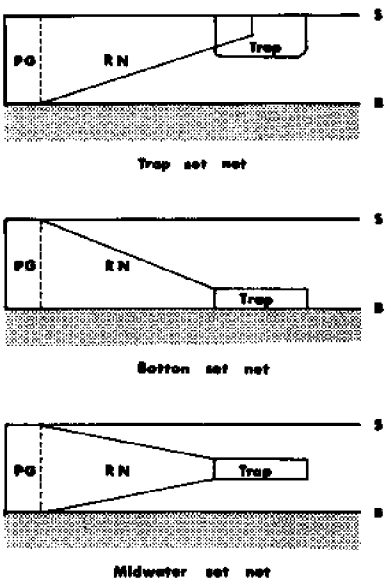

Figure 10. Schematic diagram of vertical section of salmon set nets on the Japanese coast.
S: Sea Surface
B: Sea Bottom
PG: Playground
RN: Ramp Net

dients for ramp nets are indicated in Table 3 for every 18 m of sea depth. The gradients vary from 0.12 to 0.40 and increase with increments of the sea depth. The values of gradients are similar between the trap set and the bottom set nets, because in the vertical views the bottom set net turns the trap set net upside down. In the midwater set net, ascending gradients are smaller than descending but both gradients are less than those in the other two nets.

In any case, the slope of the ramp net is steeper than the mean gradients in vertical movements of free swimming salmon. Most of the slopes in ramp nets are steeper than the mean swimming gradients when salmon are escaping the research vessel. Telemetric records on swimming depth indicate that fish entering the set nets continue to repeat upward and downward movement inside the playground and ramp nets. When fish stay in the upper portion of the ramp net in the trap set net, or in the lower portion of the ramp net in the bottom set net, or in the middle portion of the ramp net in the midwater set net, they can easily be conducted into the trap. When fish encounter the slope of the ramp net, however, it is possible they do not move along the slope because of its steep gradient. In particular, the midwater set net is unfavorable for conducting fish to the trap from the structure of the ramp net. The ramp net in the midwater set net has two slopes. Such a disadvantage in the structure of the net may relate to the fact that about half of the fish in the playground escape from the net to open water.

Although it is ideal to decrease the slope of the ramp net, the slope is controlled by the size of the set net and the bottom topography in which the set net is established. The size of salmon set nets along the Hokkaido coast is determined by regulation. One method is suggested to eliminate the barrier resulting from the steep slope of the ramp net. As shown in Table 4, the gradients of vertical movement in escapement from the vessel are larger than those when the salmon is free swimming in the natural state. Not only in escaping from restraint but also in escaping from threat, salmon exhibit sharp gradients in vertical movement. Sonic stimuli will be effective to frighten fish and conduct them toward the opposite direction from the noise.

Table 4. Descending and ascending gradients of free swimming chum salmon on the Okhotsk coast.

Horizontal moving speed (A) m/min — cm/sec		Vertical moving speed (B) m/min — cm/sec			B/A		
		Descent	Escapement	Ascent	Descent	Escapement	Ascent
Minimum	—	1.00	0.80	1.00	0.021	0.016	0.021
	—	1.67	1.33	1.67	(1.2)	(0.9)	(1.2)
Average	47.35	5.12	11.36	5.40	0.108	0.240	0.114
	78.92	8.53	18.94	9.00	(6.2)	(13.5)	(6.5)
Maximum	—	25.00	28.50	30.00	0.528	0.602	0.634
	—	41.67	47.50	50.00	(27.8)	(31.1)	(32.4)
Number of measurements	432	1694	9	1613	1694	9	1613

Parenthesis indicates angle. Escapement shows descending state just after fish was released at sea.

We have no precise information on behavior of chum salmon in the ramp net, but an ultrasonic transmitter recently developed by us makes it possible to study the response of salmon against the net. The accuracy of depth measurement is improved to ± 1 percent with the new telemetric system. Until the behavioral study is completed, conclusive statements cannot be made on evaluating the structure of the net.

Summary

Application of a tiny pressure sensor to the ultrasonic biotelemetry improved our efforts in studying the behavioral habits of salmon. Results from mature chum salmon through field

research on the coast of Japan have revealed characteristic patterns of vertical movement of salmon. When the horizontal moving speed is combined with the vertical moving speed, we can calculate the gradients in upward and downward movement for mature chum salmon in the coastal area. These gradients may be applied for improving the structure of set nets. Although the salmon set net fishery is a traditional and passive fishing method, regulation of the fishing period is needed in order to increase the activities of hatcheries. Promotion of catch efficiency by set nets in a limited fishing season must lead to better regulation of salmon aquaculture. From the field research carried out on 12 mature chum salmon along the Okhotsk coast in October of 1979, results are summarized as follows.

1. The mean horizontal ground speed was 47.4 m/min while the maximum speed was 260 m/min. There was no remarkable difference between ground speeds in the daytime and at night nor between flood and ebb tide.
2. During 43.9 percent of all periods of experiments, mature chum salmon stayed in the 0 to 5 m depth. The mean swimming depth was 13.7 m. There was no remarkable difference in the swimming depths between daytime and at night.
3. Mature chum salmon often came to the subsurface of the sea. The frequencies per hour in which salmon came up to the 0 to 5 m layer of depth were compared among four different localities on the Japanese coast. The value was 1.7 off Etorohu Island and 7.6 off the Fukushima Prefecture. Such a characteristic vertical movement probably relates to the olfactory function of maturing chum salmon.
4. In upward and downward movements, the mean vertical speed of chum salmon was 5.4 m/min and 5.1 m/min respectively. In downward movement escaping from restraint, the mean vertical speed of fish was 11.4 m/min, faster than that in the natural condition.
5. From horizontal and vertical speeds of fish, the gradients in upward and downward movement were calculated. The gradient in escapement was 0.24 while that in the natural vertical movement was 0.11.
6. The slopes of ramp nets were measured from 153 salmon set nets established along the Hokkaido coast. All slopes of ramp nets of various types exceeded the gradients in the natural vertical movements of fish. This difference in gradients may decrease catching efficiency of salmon set nets, but precise behavioral study is needed to evaluate the present structure of set nets.

Acknowledgement

We are much indebted to the Abashiri Fishermen's Cooperative Association which actively collaborated with us in offering a research vessel and other facilities. The cooperation and assistance of K. Tokunaga, K. Hachiya, M. Kiritani and other undergraduate students in our University are gratefully acknowledged. Nitto Net Manufacturing Co. Ltd. in Tokyo kindly provided us the data on structure of salmon set nets. This project received partial financial support from the Fisheries Agency in Japan.

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Downstream Migration and Seawater Adaptability of Chum Salmon (*Oncorhynchus keta*) Fry

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Abstract

All mature chum salmon which ascend rivers in Japan are collected for artificial propagation. The fry are reared in a hatchery until they reach a body weight of over 1 g; they are then released into the river. In the Otsuchi River, fry weighing 0.4 to 1.8 g had a plasma Na^+ concentration of 130 to 140 mmol/l. The greater part of them began downstream migration immediately after release and reached the estuary within 24 hours. Throughout the estuarine section, chum fry were always found in the upper low salinity layer (2 to 25 parts per thousand). Upon reaching this area, their plasma Na^+ level increased slightly to 150 to 160 mmol/l.

When the fish, weighing 0.7 g, were transferred directly from freshwater to seawater under artificial conditions, the plasma Na^+ concentrations increased markedly after one hour, reached a maximum after three to 12 hours, and attained the seawater-acclimation level of 155 mmol/l within 24 hours. The large fish, weighing 1.8 and 2.2 g, showed very high levels and the concentrations remained high 24 hours after the transfer. The osmoregulatory abilily of chum fry in seawater decreased gradually with an increase in body weight in the rearing period in freshwater.

Introduction

Chum salmon (*Oncorhynchus keta*) is the most important salmon in Japan. The mature salmon migrate to their native river close to the sea to spawn during October to January in northern Japan. We collect all of the mature fish in the river for artificial fertilization. This is to increase the population of the chum salmon. From the natural spawning ground, the young migrate to the sea as alevins or fry soon after yolk absorption. This takes place at the same stage as with pink salmon (*O. gorbuscha*), but one or two years earlier than with chinook (*O. tschawytcha*), sockeye (*O. nerka*), coho (*O. kisutch*), masu (*O. masou*) and other salmonoids (Tyler and Bevan, 1964; Hoar, 1951; Kobayashi and Abe, 1977).

Chum and pink salmon fry migrate downstream to the sea during April and May in Hokkaido (Kobayashi and Harada, 1966; Kobayashi, 1968). A few observations from the water surface explain the relationships between current speed, rheotaxis, schooling, swimming depth, and response to light (Neave, 1955; Hoar, 1954). Distribution in the estuary was investigated by seine net, and found to relate to the tidal cycle (Mason, 1974). There has been no underwater observation of fry behavior, though it is more important.

It has been generally accepted that chum alevin and fry exhibit a sharp increase in salinity resistance in the early stage (Kashiwagi and Sato, 1969). It was difficult to maintain the fry in freshwater after the migratory season. They exhibited consistent seawater preference for several months until all animals died by November (Baggerman, 1960; Kubo, 1953). There is no landlocked population of chum salmon. Therefore, we can estimate that chum salmon

acquire seawater adaptability soon after yolk absorption and that they must migrate to salt water until water temperatures in river and coastal waters exceed 15°C or seawater adaptability decreases after the migratory season.

Hiyama, et al., (1972) reported that the large-sized chum fry are better able than small fry to evade natural predators in the river during seaward migration. Since this study, it is prevalent in Japan to prolong rearing in the hatchery to increase fry size. In recent years feeding of artificial foods in the hatchery has become an important enhancement technique for rearing chum fry in Japan. However, there is no information on changes in seawater adaptability of the chum fry reared in freshwater for long periods. The investigation being reported was carried out to determine the relationship between seawater adaptability of fry, their size, and time spent in freshwater. Their migratory behavior is discussed in relation to their changes in seawater adaptability.

Materials and Methods

The Otsuchi Salmon Hatchery is located 1.7 km upstream from the mouth of the Otsuchi River. Fry weighing 0.4 to 2 g were transported 500 m downstream from the hatchery and released into the Otsuchi River. About 27 million fry migrated seaward in late March and April in 1977 to 1980. Observations were carried out underwater by snorkeling and scuba diving in the river and estuary. Temperature, salinity and water flow speed were measured in each investigation. A trap net was set at the end of Gensui Creek, a branch of the Otsuchi River, to count fry migrating downstream and fish remaining in the creek. The fish were caught by cast net or trap and held for at least 24 hours in water at the spot where the fry were caught.

Three groups of fry (A, B, and C) were reared in freshwater at the Otsuchi Marine Research Center, University of Tokyo. The fry were transferred directly into seawater at different growth stages after rearing for a period of time in freshwater. Blood was collected from the caudal artery by the method of Jozuka and Adachi (1979). Plasma was separated by centrifuging at 10,000 rpm for ten minutes at room temperature. Sodium concentration of the plasma was measured by atomic absorption spectrophotometer (Hitachi 170-50 or Hitachi 203) after 1,000-fold dilution with deionized water.

Results

Behavior of Migrating Fry in the River

Chum salmon fry were transferred from hatchery ponds to a water tank on tracks, transported 500 m to the Otsuchi River, and released at 1.2 km upstream from the river mouth (Figure 1). Release of fry began in late March when the water temperature in Otsuchi Bay increased to about 7°C, which was similar to the 6° to 9°C water temperature of the Otsuchi River. By late April, release of fry was finished. Two or three million fry were released into the river during daylight on several days. The first fish to be released during the day were traced and observed underwater.

Throughout the 1,700 m length of the investigated stream, water flow speed fluctuated from over 100 cm/sec in rapid current to -20 cm/sec downstream at high tide. Swimming behavior of fry schools corresponded to current speed. After release into the river, the fry lay on the bottom and did not respond to a water flow of 15 to 20 cm/sec, behavior of observer, or any other stimuli for the first three minutes. The fry were observed to twitch and their head directions were random. They began swimming motions and formed schools three to five minutes after their release. In water moving less than 20 to 30 cm/sec, the fry schools started downstream, along the bank, with their heads directed seaward. However, in currents of 30 to 80 cm/sec, their heads were oriented upstream and they were displaced by the water flow.

In a shallow, rapid flow, 80 to 120 cm/sec, their position became random due to the strong water turbulence.

During migration, the fry schools moved downriver through the bottom layer, and they were strongly attracted by shade or darkness of waterweed communities, deeps, big stones, and artificial structures. Fry schools stopped often at these shady spots (Figure 2). They

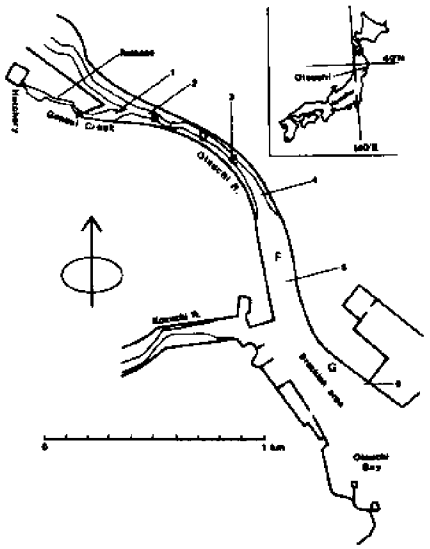

Figure 1. Map showing study area of the Otsuchi River and Gensui Creek in northeastern Honshu, Japan. Chum salmon fry released from hatchery; location of counting trap in Gensui Creek (1); sampling sites for blood collection in freshwater stream and brackish water area (1-6). Fry were caught by cast net within 24 hours after release and transported to laboratory in water taken from the spot where fish were caught.

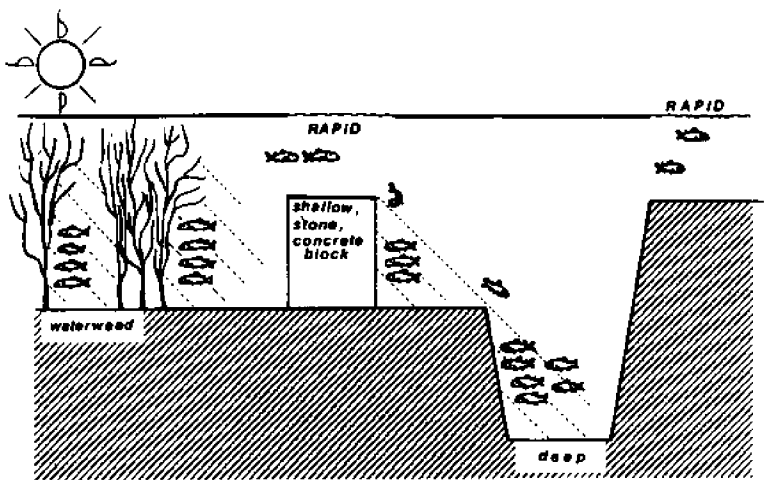

Figure 2. Schematic representation of relation between circumstances in freshwater stream and remaining sites of chum salmon fry or negative rheotaxis after release. The fish schools were strongly attracted by waterweeds, big stones and depths. They remained in the shade; feeding behavior was observed. Released fry migrated downstream in flat bottom, sunny areas.

would discontinue migration and remain in the shade. When they reached this shade, all individuals were still for a short time and changed from a stationary to an active state. They began their feeding with quick movements. A few individuals showed an investigative behavior around the shaded areas and other fish followed them. When the density of fish in the shade became high, many fry pursued any investigative fry. The school then moved out from the shade and downstream.

There are many concrete blocks protecting a bridge 400 m upstream from the river mouth (Figure 1, section E). All fry displaced from upstream stopped there and remained beside the blocks. The water level and the flow speed in section E are affected by tides. The remaining fry migrated downstream at high tide on the bottom layer. Seawater came up to section F on the river bottom where the mixing of freshwater and seawater appears. The fish responded strongly to the mixed water and turned back to freshwater or swam up to the upper low salinity layer.

Estuary Behavior

During migration through the freshwater region, the fry prefer the bottom layer. However, once the fry encountered saline water in section F or G, they changed their swimming layer to the upper (lower) salinity layers.

In the brackish area, section G, chum fry were observed along the northeastern shore. Mixing of freshwater and seawater is not complete throughout sections F and G, and the irregular refractions of light disturbed observations and measurements of salinity. Fry schools discontinued their migration on reaching the mixed water region, and the fish became immobile during observations. Fry hung near the edge of the bank, but the water flow slowly carried the fry to high salinity water outside of section G. Chum fry in this upper low salinity layer were stressed by their excited action on the surface. The fish dove quickly from the surface to deep areas. However, when the fish arrived at the saltwater boundary, they immediately returned to the upper low salinity layer and escaped the threat from the upper layer.

Remaining Freshwater Population

In section A, Gensui Creek, many fry remained for a long period after the natural migration season. A trap was set at the end of section A on 11 April 1978, at the midpoint of the releasing period. From the release point in the Otsuchi River, a group of released fry migrated upstream before the trap was set and stayed. After the trap was set, the fish could not go up Gensui Creek. For 57 days, from 11 April to 7 July, 46,195 fry were trapped. The number of trapped fish from the remaining population decreased logarithmically (Figure 3).

In another experiment, 68,000 fry were fin-clipped and released upstream in section A. After the first 24 hours, 44,128 marked fry were caught by the trap, 43,206 of which were fin-clipped (98 percent of the recaptured fry). A total of 35 percent of the fish were lost in Gensui Creek.

The trapped fish (mean body weight, 1.9 g) which remained for at least 38 days in section A were transferred directly into seawater (8°C, 33.5 ppt) to examine their seawater adaptability. Control samples (mean body weight, 0.62 g and 0.68 g) from the hatchery were examined by the same procedures. Another sample (mean body weight, 2.1 g) caught in Otsuchi Bay was transferred into seawater. Three control samples exhibited a 95 percent survival rate during the first 24 hours. Thirty percent or less of the remaining population survived the direct transfer into seawater (Figure 4).

Plasma Sodium Levels During Seaward Migration

The chum fry (mean body weight, 1.03 g) were released experimentally from the hatchery 1,700 m upstream on the Otsuchi River. They were recaptured at five sites in the freshwater

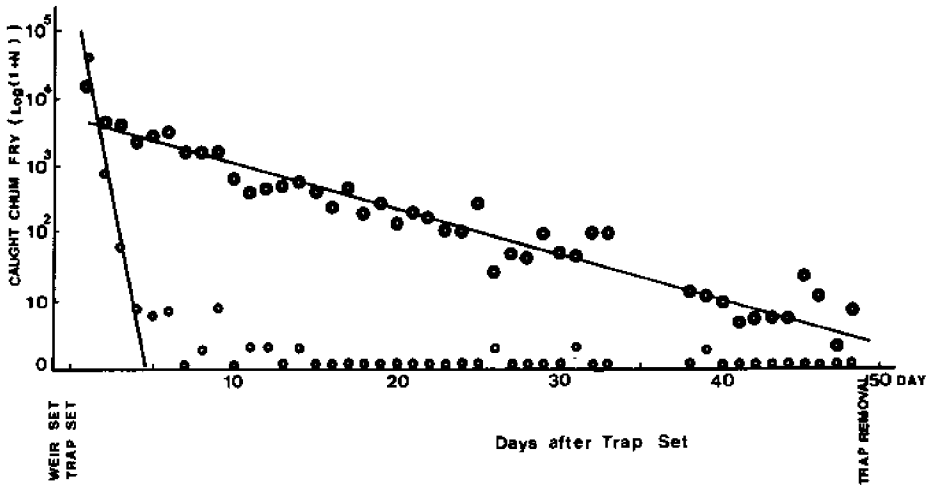

Figure 3. The progress of chum salmon fry migration from Gensui Creek to the Otsuchi River during 11 April to 7 June 1978. Small circles show the number of trapped fry which were fin-clipped and released at 400 m upstream from the counting trap. Large circles represent the number of fish that migrated up Gensui Creek from the releasing point in the Otsuchi River and migrated down again. The remaining population decreased logarithmically for 57 days.

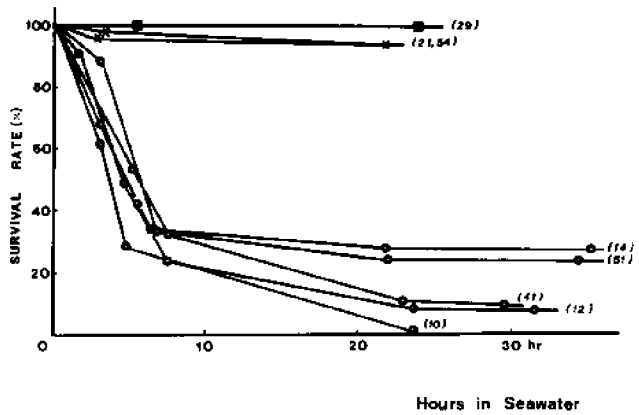

Figure 4. Seawater tolerance (8°C, 33.5 ppt) of chum salmon fry remaining in freshwater stream for at least 38 days after release (o); hatchery-reared fish (x); fry captured in Otsuchi Bay (•). Number in parentheses shows sample size.

section and two sites in the brackish area. The fish seemed to be under stress after being transported from the river to the laboratory, and their plasma Na^+ concentrations were highly variable. Therefore, the fry were kept in water from the collecting spot for at least 24 hours before blood sampling. As shown in Figure 5, plasma Na^+ concentrations of the fry sampled from the river were 134 to 140 mmol/l, and there was no significant difference among the five shoals regardless of migration distance from the hatchery. The fry were also caught in the estuary 24 hours after release, and the plasma Na^+ concentrations (151 to 153 mmol/l) were significantly higher ($p < 0.01$) than those in the river, almost the same as in the fry adapted to seawater.

Relationship Between Seawater Adaptability and Body Size

Seawater adaptability of fry of different sizes was examined simultaneously in late April.

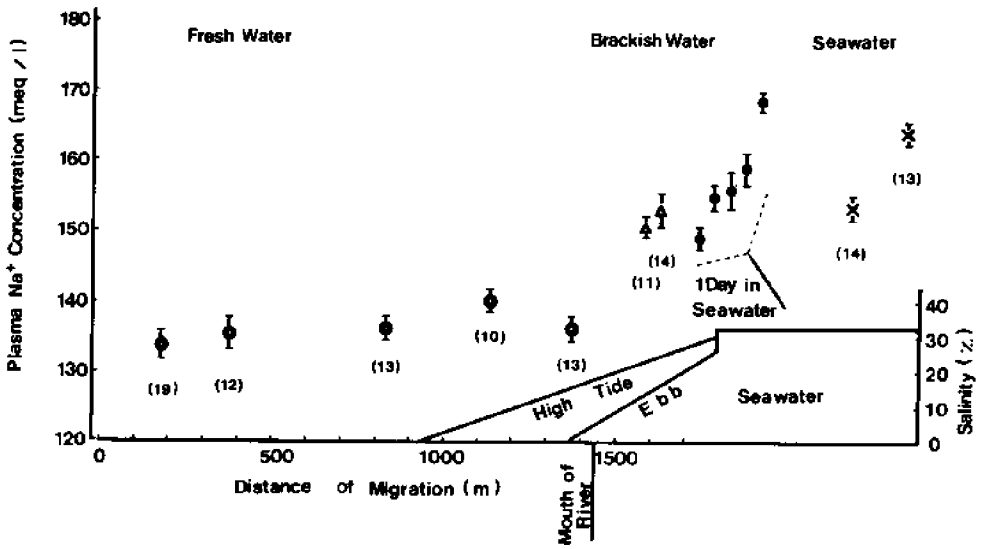

Figure 5. Plasma Na^+ concentrations of chum salmon fry caught in the river (o), estuary (△) and bay (x), and of fry 24 hours after transfer from freshwater to seawater in the laboratory (•). Vertical bars represent standard errors (numbers of fry in parentheses). Salinity is shown schematically at bottom right.

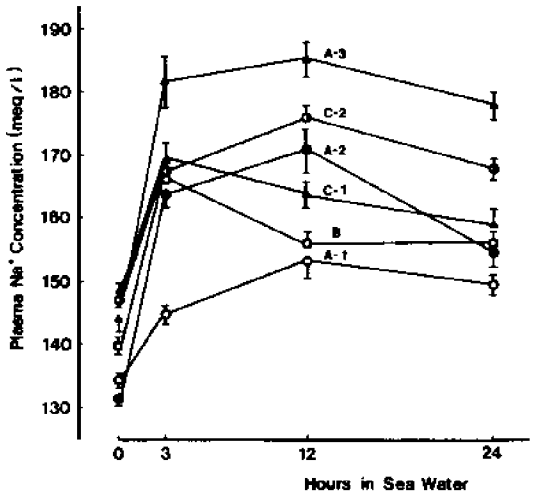

Figure 6. Changes in plasma Na^+ concentrations of chum salmon fry after transfer from freshwater to seawater. Fish of Group A and B were reared from April to July and examined.

As shown in Figure 6, plasma Na^+ concentrations of the smallest fry (Group A-1, mean body weight, 0.41 g) increased from 136 mmol/l in freshwater to 146 mmol/l after three hours in seawater, and to 154 mmol/l after 12 hours. This group attained the seawater-acclimated level within 12 hours without showing any sharp peak, although there was a tendency toward a decrease of Na^+ after 24 hours. A small amount of yolk still remained in some of the fry from this group. In nature, fry at similar stages are known to start seaward migration. Fry weighing 0.74 g (Group B) showed a peak in the Na^+ level three hours after transfer, and attained the seawater level after 24 hours. Plasma Na^+ concentrations of the large group (Group C-1), weighing 1.26 g, similarly reached a peak after three hours. The level after 12 hours was still high.

In another experiment, changes in seawater adaptability of the same fry (Groups A and C) were examined until five months after hatching. Group A fry were reared for about two months and weighed 1.43 g, and grew to a mean weight of 1.76 g at six months after hatching (Group A-3). The Na^+ concentration reached 183 mmol/l after three hours and still remained high after 24 hours. The level of Na^+ concentration of the fry in Group A-3 was raised for the longest period. They reached 183 mmol/l after three hours and still remained high after 24 hours. During the 24 hours in seawater, the plasma sodium level of Group A-3 was 10 to 20 mmol/l higher than Group A-2. The same tendency was observed in the fry of Group C. In the largest fry (Group C-2), weighing 2.25 g, the highest concentration was 176 mmol/l and occurred after 12 hours, and was maintained after 24 hours.

Discussion

In Japan, the chum salmon, *Oncorhynchus keta*, is especially abundant in the water of Hokkaido and the northern part of Honshu. In the Sanriku area where the Otsuchi Marine Research Center is located, mature fish return to their natal river during October to January. Artificial propagation of the chum is common in this area. The emerged alevins are reared in the hatchery until they attain an average body weight greater than 1 g and are then gradually released into rivers from March to May. The seaward migration, of the native fry whose yolk still remained in some of the fish, was earlier than that of the artificially reared population.

According to Kobayashi and Abe (1977), alevins and reared fry, unfed for a month in the hatchery, migrated downstream 18 km on the releasing day. A small number of fry were observed that had remained in the river for 1.5 months after release. Similar behaviors were observed in the present study. More than 98 percent of the fry recaptured by an experimental trap migrated 400 m downstream during the 24 hour period following release. A few different groups of fry migrated upstream from the releasing point before the trap was set and remained there for a total of 48 days. Tyler and Bevan (1964) showed a small group of chum fry in Nooksack River, Washington, remained until late June.

In the downstream migration group and the remaining group of fish, the fry were strongly attracted by the shade or darkness of waterweeds, deeps, big stones and artificial structures. Hoar (1958) examined the hiding behavior of four Pacific salmonids and explained it in relation to the advantages of predation avoidance. When chum fry found observers on the bank or underwater, they exhibited hiding behavior by retreating to waterweeds or shade.

Hiyama, et al., (1972) found that chum fry tagged by ^{60}Co or ^{198}Au pins were eaten by fishes, *Cottus hangiongensis*, *Chaenogobius urotaenia* and *Tridentiger obscurus*. During the trap experiment in this study, a total of 42 chum fry were found in the stomachs of two trout (*Salmo gairdneri*). The downstream migration behavior pattern (a preference for river shade and remaining in waterweeds or deeps) may be advantageous in avoiding predators.

In freshwater the fry migrating downstream preferred the bottom layer in daytime; but once the fry encountered saline water in the estuary, they began swimming in the upper and lower salinity layer, even in strong light. This change in swimming layers suggests that seawater adaptation is not easy for chum salmon fry. The fish in brackish water were sluggish and avoided diving into the seawater layer. They seem to evade a sudden change in their environment of salinity and osmosis.

Plasma sodium concentration of the fry sampled from the river were 130 to 143 mmol/l. The concentrations (151 and 153 mmol/l) in the fish groups caught in brackish water 24 hours after release reached the same level as in the fish adapted to seawater. Chum and pink salmon acquire salinity tolerance earlier in life as compared with other salmonoids (Parry, 1960; Conte and Wagner, 1965; Conte, et al., 1966; Weisbart, 1968; Kashiwagi and Sato, 1969; Kobayashi and Harada, 1966). As shown in the present study, when transferred directly to seawater, chum fry are able to adjust plasma ion levels within 24 hours. Plasma sodium con-

centration of the smallest fry, still containing a small amount of yolk, showed a gentle increase without any sharp peak and attained the seawater-acclimated level within 24 hours. Other larger fry groups had a tendency toward a decrease of seawater adaptability. The present data are in contradiction to the previous findings of Houston (1961) that 1.6 g fry were better able to regulate total body chloride levels after seawater transfer than were 0.9 g fry. It is unlikely that this difference is due to the fact that different methods were employed to examine the seawater adaptability. The chum fry, in the present study, were kept in the laboratory aquarium at 10°C for more than two weeks before being transferred to seawater (10°C, 33.5 ppt). When fry of different lots were transferred to seawater simultaneously in late April, the smaller fry adjusted their plasma Na^+ levels more easily than the larger fry. Also, when seawater adaptability of the same lots of fry were followed until five months after hatching, the osmoregulatory ability of the fry in seawater decreased gradually with an increase in body weight and correlates with the time spent in freshwater. The data suggest that to keep chum fry in a hatchery for a long period in order to increase their size is not advantageous where smooth adaptation to seawater is concerned.

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The Distribution and Residency of Juvenile Pacific Salmon in the Strait of Georgia, British Columbia, in Relation to Foraging Success

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Abstract

This report summarizes information on the distribution and residency of juvenile salmon in the Strait of Georgia, British Columbia, and emphasizes circumstances in which distribution and residency appear to be related to foraging success. Occupation of estuaries and nearshore areas by pink, chum, and chinook fry during April and May appears not to be related to foraging success, nor does their movement away from shore in May and June, with the possible exception of the movement of chum out of rivermouth habitats. Departure of pink, chum, and sockeye from the Strait of Georgia in July, and the distribution of coho, chinook, and chum within the Strait in late summer, however, do appear related to foraging success.

Introduction

Mortality of Pacific salmon during the first few months after they enter the sea is thought to be high, and possibly critical to adult returns (Parker, 1965 and 1968). Causes of mortality at this time can only be conjectured, but are considered to be intimately related to growth rate (Parker, 1971) and presumably reflect the carrying capacity of ocean habitats in some way. Attempts to enhance salmon production through the release of large numbers of fry or smolts may result in local or general overexploitation of food resources by the fish, or elicit a functional response of marine predators (Peterman and Gatto, 1978) so that no net gain in salmon production results. An understanding of the ecological relationships affecting young salmon during their early sea life, therefore, is essential to a full understanding of variations in stock abundance, and to any assessment of the capacity of the ocean to produce more salmon.

In this report I shall present information on the distribution and abundance of Pacific salmon in the Strait of Georgia, an important marine nursery area for salmon in southern British Columbia. I shall emphasize situations in which distribution, and residency in some habitats within the Strait could be a function of foraging success. The observations summarized here are drawn from previously published (Healey, 1979, 1980a and 1980b) and unpublished information on the ecology of juvenile salmon in the Strait of Georgia.

The Strait of Georgia

The Strait of Georgia, a partly enclosed body of water 220 km long by 33 km wide (surface area 6900 km^2), lies between the southern end of Vancouver Island and the mainland shore of

British Columbia and Washington State (Figure 1). Greatest biological productivity is in the southern half of the Strait, and is influenced by tidal mixing, entrainment in the Fraser River plume, and turbidity in the surface waters (Parsons, et al., 1969a; Stockner, et al., 1979). Zooplankton blooms in the surface waters occur from March to May in the southern half of the Strait, and produce standing crops in excess of 1,000 organisms/m³. In the central and northern parts of the Strait zooplankton blooms are generally later and standing crops smaller (Hutchinson and Lucas, 1931; Stephens, et al., 1969; Parsons, et al., 1969b; Stockner, et al., 1979). Zooplankton biomass in the surface waters of the Strait of Georgia, in general, increases through March and April, peaks in May, and declines in June. In some parts of the Strait zooplankton biomass may remain high throughout August and September, but small zooplankters dominate after June (Stephens, et al., 1969; Mackas, et al., 1980).

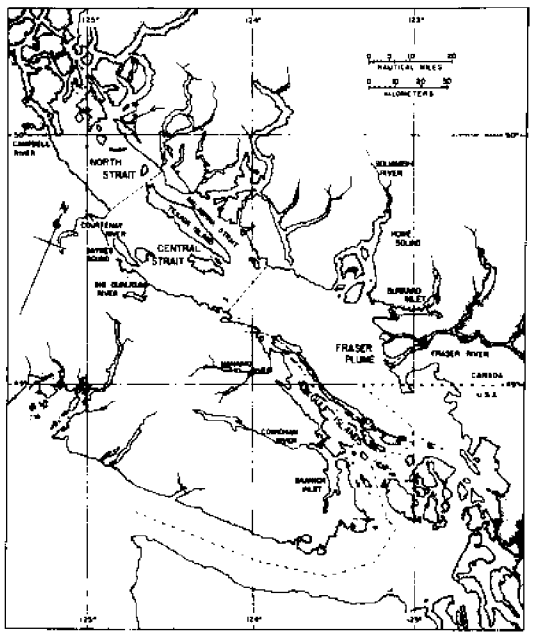

Figure 1. Map of the Strait of Georgia showing rivers and major landmarks referred to in the text and the four regions of the Strait between which comparisons of abundance and feeding were made.

The principal salmon rivers tributary to the Strait of Georgia within Canada include the Fraser and Squamish rivers on the mainland shore, and the Cowichan, Nanaimo, Qualicum, Courtenay, and Campbell rivers on the Vancouver Island shore (Figure 1). During the period 1965 to 1975 escapements to rivers tributary to the Strait averaged about 1.2 million sockeye (*Oncorhynchus nerka*), 1.8 million pink (*O. gorbuscha*) (odd years only), 850,000 chum (*O. keta*), 230,000 coho (*O. kisutch*), and 100,000 chinook (*O. tschawytscha*) annually. Most of these fish spawn in the Fraser River (Aro, et al., 1977). The input of juvenile salmon into the Strait of Georgia each year is substantial; on the order of 300 million in odd years, and an additional 200 million pink fry in even years.

Seasonal Patterns of Distribution and Abundance

Chum and pink salmon fry enter the Strait of Georgia as recently emerged fry between March and May each year. During their first few weeks of ocean residence they congregate

close to shore in water only a few cm deep. They occupy these very shallow waters until early June. The habitats utilized by the two species are similar, except that pink fry are not abundant in river delta areas while chum are. By early May some of these fish have begun to move away from the shallow beach areas to rear over deeper water, and by June both species are abundant in purse seine sets over deeper water (20 to 40 m). Purse seine catch of these two species in the Strait of Georgia declines during late June and July, and by August few are captured in the Canadian waters of the Strait (Figure 2, Table 1) (Healey, 1980b; Barraclough and Phillips, 1978).

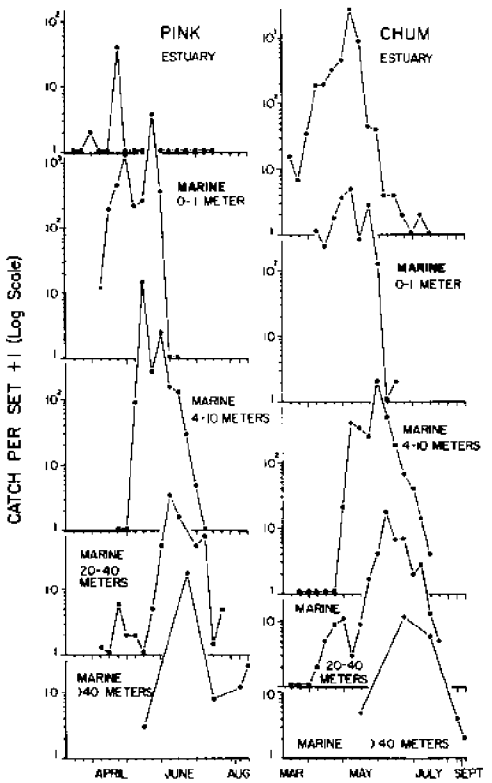

Figure 2. Seasonal abundance of chum and pink salmon in waters of various depths near Nanaimo in the Strait of Georgia (from Healey, 1980b).

Table 1. Catch-per-set of juvenile salmon throughout the Gulf Islands region of the Strait of Georgia, May-October 1976 and in the vicinity of Nanaimo, April-December 1976.

	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
					Gulf Islands Region				
No. sets	0	40	40	23	45	40	44	0	0
Chum	—	4.5	140	71	2.8	1.3	1.8	—	—
Pink	—	1.6	660	7.4	11	23	0.3	—	—
Coho	—	6.0	1.3	5.9	5.2	6.0	1.2	—	—
Chinook	—	0.0	1.7	2.1	2.0	2.8	1.7	—	—
Sockeye	—	6.3	7.3	1.4	0.0	0.02	0.1	—	—
					Nanaimo				
No. sets	73	91	54	45	0	7	0	16	4
Chum	2.5	13.5	61.6	6.9	—	1.3	—	0.00	0.00
Pink	2.0	9.0	126.7	16.3	—	0.14	—	0.00	0.00
Coho	0.12	0.13	1.6	2.4	—	2.1	—	1.9	0.00
Chinook	0.00	0.00	0.90	2.6	—	3.6	—	1.7	0.75
Sockeye	0.00	7.2	5.7	0.00	—	0.00	—	0.00	0.00

Virtually all sockeye salmon juveniles entering the Strait of Georgia come from the Fraser River. Most migrate to sea in April and May as yearling smolts, but some migrate seaward as fry and rear in the Fraser River delta until July or August. During April and early May yearling sockeye smolts are concentrated in the Fraser plume area near the mouth of the Fraser River. During late May and June the main concentration of yearling smolts moves south and west into the Gulf Islands region of the Strait. Catch in the surface waters of the Strait declines in July and few yearling sockeye are captured in August (Barraclough and Phillips, 1978; Healey, 1980b) (Table 1). Underyearling sockeye smolts apparently enter the Strait of Georgia in July, and concentrations of these fish occur off the mouth of the Fraser River, and in the entrance to Howe Sound. Underyearling sockeye are rare relative to the yearling smolts, however, and their length of residence in the Strait of Georgia is unknown.

The decline in catch of pink, chum, and yearling sockeye juveniles in June and July in the Strait coincides with an increase in the abundance of these species on the outer coast (Hartt, 1980). It seems reasonable to conclude, from the coincidence of these events, that July is the principal month of outmigration of these three species from the Strait of Georgia. The completeness of the outmigration appears to vary from year to year, however, and several authors have reported considerable numbers of pink, chum, and sockeye still resident in parts of the Strait and other inside waters after July (Hartt, 1980; Healey, 1980b; D. Blackbourne, pers. comm.).

Coho salmon enter the Strait of Georgia as yearling smolts during April and May from virtually every tributary stream. Catches in the Gulf Islands region and near Nanaimo remain relatively constant from May through October with no indication of a substantial outmigration before winter (Table 1; Healey, 1980b; Schmidt, et al., 1979).

Chinook salmon migrate seaward as recently emerged fry from March to May, as underyearling smolts in June, and as yearling smolts from March to May of their second year (Healey, 1980a, and unpublished data; Reimers, 1971; Rich, 1920). In the Strait of Georgia, fry migrants rear in marshes and intertidal channels at the river mouths where salinity ranges up to 24 parts per thousand. They disperse away from these habitats into higher salinity areas of the Strait in June, coincident with the downstream movement of underyearling smolts from the rivers. Catch of underyearling chinook in the open waters of the Strait remains rather constant throughout July to November and declines during the winter (Table 1; Healey 1980a and 1980b). Yearling smolts are common in seine catches in the Strait of Georgia only during May to July after which they disappear from the Strait (Healey, 1980a and 1980b).

Relationships with Foraging Success

The above description of the early sea life of salmon demonstrates that they are essentially transients in any of the habitats that they occupy. Several major redistributions of fish, such as the movement of pink and chum away from the beaches in May, the movement of chinook out of estuaries in June, and the movement of pink, chum, and sockeye out of the Strait of Georgia in July, can be recognized. These redistributions appear to be size-dependent, the larger fish tending to move first (Healey, 1980b). While the seasonal timing of these movements could be innate, and related to age or ontogeny, there appears to be sufficient flexibility in the timing and completeness of each redistribution to suggest that features of the local environment stimulate the movement of fish. In particular there is evidence that occupation and abandonment of a particular habitat is, in some instances, related to foraging success in the habitat.

In the Nanaimo River estuary residence of chum fry was related to the seasonal abundance of their principal prey species, *Harpacticus uniremis*. Estimates of the amount of *H. uniremis* eaten by the chums were of the same order as estimates of *H. uniremis* production, leading to

speculation that the abundance of chums was potentially limited by the production of this copepod (Healey, 1979; Sibert, 1979).

Comparable data are not available for other nursery areas. Data on stomach content as a percent of body weight are available, however, and provide a rough index of foraging success. Stomach content of pink, chum, and chinook salmon captured in the Nanaimo area was compared between habitats (river mouth, high salinity nearshore, and offshore habitats) for three time periods (prior to 15 May when the main concentration of fish was in river mouth and high salinity nearshore habitats, 15 May to 15 June when pink and chum disperse from river mouth and nearshore habitats to offshore habitats, and after 15 June when pink and chum are dispersing rapidly out of the Strait, but chinook are not). Data for chum are from 1975 and 1976, for pinks from 1976 only, and for chinook from 1975 to 1979. Average stomach content differed significantly between years for both chum ($F = 4.947$, df 1/243) and chinook ($F = 8.876$, df 3/51), but the seasonal pattern did not differ between years. The data were, therefore, averaged over years to emphasize the seasonal pattern in the different habitats (Table 2).

Table 2. Average stomach content, as percent body weight, of juvenile chum, pink, and chinook salmon in different habitats of the Strait of Georgia during river mouth and nearshore residence (prior to 15 May), offshore movement and residence (15 May-15 June) and the outmigration of pink and chum salmon from the Strait of Georgia (after 15 June). Temperature range observed during sampling in the different habitats is also shown.

	Rivermouth			Nearshore			Offshore		
Species	< 15 May	15 May-15 June	> 15 June	< 15 May	15 May-15 June	> 15 June	< 15 May	15 May-15 June	> 15 June
Chum	2.10	3.25	1.84	2.36	3.40	—	2.06	2.88	1.48
Pink	—	—	—	1.52	2.80	—	1.73	2.45	1.10
Chinook	1.73	1.98	1.91	—	—	—	—	1.70	1.59
Temp. Range °C	8-18	10-22	15-24	8-13	10-17	—	7-11	10-13	13-17

Feeding pink and chum fry may commonly be observed taking advantage of a variety of natural food delivery and concentrating mechanisms, such as eddys and current shear lines, in the habitats they occupy. Shallow nearshore habitats should be characterized by high food densities for a variety of reasons. In spring, temperature can be relatively high in shallow water so that food organisms increase in abundance and are more active than in cooler offshore waters. The small crustacea on which the fry feed are ten to 20 times more abundant in the 10 cm of water just above the bottom (Sibert, 1980) and in shallow water the fish can take advantage of any tendency of prey to concentrate at either the bottom or the surface. Stomach content data, however, do not confirm that foraging is more successful in nearshore habitats in spring. Chum stomach contents ranged from 2.06 to 2.36 percent of body weight and were slightly greater in nearshore habitats than offshore, but the difference was not significant ($F < 1.0$). Stomach content of pinks ranged from 1.52 to 1.73 percent of body weight and was lower in nearshore waters than offshore, but again the difference was not significant ($F < 1.0$). Chinook young-of-the-year are restricted to estuaries at this time of year, a distribution that is probably determined by their physiology rather than food resources (Healey, 1980a). The stomach content of chinook in the estuary averaged 1.73 percent of body weight.

The 15 May to 15 June period appeared to be a time of relatively good foraging for chum and pink juveniles in all the habitats sampled. Stomach contents of chum were from 2.88 to 3.40 percent and of pink were from 2.45 to 2.80 percent of body weight, significantly higher in all habitats compared with before 15 May ($F = 12.6$, df = 1/236; $F = 6.09$, df = 1/83 for chum and pink, respectively). Again there was no significant difference between habitats,

although stomach contents of fish captured offshore were slightly lower than nearshore and rivermouth habitats (Table 2). Chinook stomach contents were from 1.70 to 1.98 percent of body weight, unchanged from before 15 May.

After 15 June pink and chum stomach contents were the lowest recorded, 1.10 to 1.40 percent of body weight in offshore habitats, significantly lower than the 15 May to 15 June period. There was no significant change in the stomach contents of chinook (Table 2). Water temperature was higher after 15 June (Table 2) and this would result in somewhat lower average stomach content, even if meal size were the same, because of more rapid digestion. The observed increase in temperature, however, would result in, at most, a 20 percent reduction in average stomach content rather than the 50 percent reduction observed. These very low stomach contents were coincident with the period of rapid migration of pink and chum out of the Strait of Georgia.

Sampling throughout the Strait of Georgia during July to September of 1975 and 1976 revealed that coho, chinook, and chum differed in abundance between regions of the Strait of Georgia (Table 3). Chum were most abundant in the Gulf Islands region in 1975 and rare in the central Strait region. Chum were less abundant throughout the Strait in late summer 1976, and the differences in abundance between regions were proportionately much less. Coho were most abundant in the Gulf Islands region and least abundant in the Fraser River plume and central Strait in both years. Like chum, coho were less abundant in 1976 than in 1975. Chinook were most abundant in the Fraser River plume region in 1975. Abundance of chinook differed little between regions in 1976, although abundance was still greatest in the Fraser River plume. Chinook were slightly more abundant in 1976 than in 1975. Stomach contents of fish in these samples did not exceed 1.73 percent of body weight and were often less than 1 percent (Table 3). Stomach content and abundance (transformed to natural logarithms) were positively correlated both within and between years for all three species. For chinook and chum the correlation overall was statistically significant ($F = 16.26$, $P < 0.05$, $F = 23.57$, $P < 0.01$, respectively), but not for coho ($F < 1.0$).

Discussion

While the data are by no means conclusive, they indicate that distribution and residence of juvenile salmon in some habitats within the Strait of Georgia were associated with foraging success. In particular, the emigration of pink and chum juveniles from the Strait of Georgia, and the regional distributions of chum, coho, and chinook juveniles in late summer were correlated with stomach contents. Occupancy of nearshore habitats by pink and chum in spring and movement away from shore in May were not correlated with stomach contents, except that the emigration of chum juveniles from the river mouth habitat of the Nanaimo estuary was related to a decline in the abundance of their principal prey.

It is not surprising that there was not a universal relationship between distribution and foraging success. The behavior of Pacific salmon at any time must always reflect some tradeoff between competing needs, as Eggars (1978 and 1980) has suggested. The principal needs of juvenile salmon appear to be: to obtain food, to avoid predators, and within these constraints, to maximize growth rate. The absence of a relationship between distribution and foraging success in spring and early summer probably indicates that food resources were adequate in most habitats to provide successful foraging. The distribution of the fish may then be controlled by another need, such as avoiding predators or taking advantage of relatively warm nearshore temperatures to maximize growth efficiency (Brett and Higgs, 1970). Growth rate of pink, chum, and chinook during their first few weeks of ocean life is very rapid (Healey, 1979, 1980a, and 1980b) suggesting that food is not directly limiting at this time.

After 15 June, when stomach contents generally declined, the fish began to show distribu-

Table 3. Catch per set, and average stomach control content as percent of body weight for chum, chinook, and coho in four regions of the Strait of Georgia, July-September 1975 and 1976.

	Chum				Chinook				Coho			
	1975		1976		1975		1976		1975		1976	
Region	Catch	Stomach	Catch	Stomach	Catch	Stomach	Catch	Stomach	Catch	Stomach	Catch	Stomach
Gulf Islands	34.6	1.25	1.45	—	2.9	1.11	3.02	—	10.2	1.38	7.05	—
Fraser Plumes	20.1	1.07	0.63	0.68	8.1	1.51	4.25	1.73	6.0	1.10	2.67	1.08
Central Strait	2.7	1.05	0.78	0.78	0.40	0.40	3.30	0.83	5.3	0.58	3.80	0.50
North Strait	14.3	1.12	1.30	0.78	0.10	—	3.40		7.2	1.55	5.80	

tion patterns related to foraging success. Presumably, available food resources were now low enough that the fish had to seek out the best feeding areas to satisfy their food requirements. For pink, chum, and sockeye, which are largely planktivorous, this meant leaving the Strait of Georgia. Coho and chinook, which are piscivorous, were able to satisfy their food requirements within the Strait of Georgia.

The capacity of the Strait to support juvenile salmon, and the impact of increasing juvenile salmon populations through enhancement, on distribution, growth, and survival, cannot be estimated directly from these observations. The indication that food resources may be limiting in late summer, however, warns that the release of large numbers of juveniles from hatcheries could overload the habitat. What the consequences of such overloading would be are uncertain. The fish might merely redistribute themselves with no failure of the expected gain in productivity. On the other hand, the possible failure of hatchery releases or the decline of wild stocks as a result of competition for scarce food resources should not be dismissed lightly.

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Natural Reproduction of the Far East Chum (*Oncorhynchus keta* Walb)

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Among all the species of the Pacific salmon the Far East chum is one of the most abundant. It has been harvested by the Soviet fleet in the coastal area of the Soviet Far East and by the Japanese fleet on the high seas of the Northwest Pacific and Bering Sea, as well as in the coastal areas of Hokkaido and Honshu in the last 25 years.

Chum stocks have suffered essential changes during this 25-year period of intensive exploitation. The aim of the present investigation is to analyze changes in harvesting and reproduction, and to understand the reasons for reproduction and stock reduction. Reports of the Soviet-Japan Fisheries Commission, Soviet-Japanese Commission on Fisheries, INPFC, scientific publications of Soviet, American, Canadian and Japanese scientists and information from the weather services were used for this investigation. Data on chum distribution in the North Pacific, including tagging data, biological materials on the marine period of life and samples in the coastal areas of the USSR, statistical data on chum catch in the sea and coastal areas, and estimation data on numbers of chum spawners on the spawning grounds, were used.

The present report deals with information on the main stocks of the Pacific chum, i.e., the chum from the rivers of the continental coast of the Okhotsk Sea, the chum from the western and eastern part of Kamchatka and the Amur River. In these areas natural reproduction is the basic type of reproduction of chum stocks. For a number of reasons the chum of the Anadyr River, Primorye territory, Sakhalin and Kuril Island were excluded from the analysis. In recent years chum from the Primorye territory have been so few that they are not estimated by the fisheries statistics and their reproduction is at a very low level. The chum from Sakhalin and Kuril Island are not distinguished in the high seas catches but are counted together with the Hokkaido and Honshu chum. The chum from the Anadyr River are not distinguished from the high seas catches at all. As we can see, these were the principal shortcomings of the distribution scheme of different chum stocks in the sea and their distinction from sea catches worked out by the Japanese scientists. The scheme is based on tagging, scale structure, biological data (length, weight, maturity, age and sex structure) and migration data from the sea fisheries research vessels.

According to the scheme, the Northwest Pacific and the Bering Sea are divided into three areas: A, B and C.

Area A: the Bering Sea. North of 56°N up to meridian 175°E is the western part of the area; to the east of meridian 175°E and to the north of 54°N is the eastern part of the area. In area A, in May and June the chum from the eastern part of Kamchatka make up 90 percent of the catch, and the chum from Sakhalin, Kuril Island, Hokkaido and Honshu is 10 percent; in July and August the chum from the western part of Kamchatka is 10 percent and the chum from Sakhalin, Kuril Island, Hokkaido and Honshu is 20 percent.

Table 1. Far East chum stock and their exploitation from 1955 to 1979 (mln. specimens).

Periods	1955-1960		1961-1965		1966-1970		1971-1976		1977-1979	
	average	%	average	%	average	%	average	%	average	%
Okhotsk continental coastal chum										
Stock	21.43		9.76		7.80		5.97		4.07	
Catch	14.83	69.2	6.92	70.9	5.06	64.9	4.64	87.7	2.40	59.0
incl. in a sea	8.78	41.0	4.30	44.1	4.02	51.6	4.40	73.7	2.07	50.9
coast	6.05	28.2	2.62	26.8	1.04	13.3	0.24	14.0	0.33	8.1
escapement to spawning	6.60	30.8	2.84	29.1	2.74	35.1	1.33	22.3	1.67	41.0
Western Kamchatka chum										
Stock	12.52		5.60		4.52		5.35		2.80	
Catch	10.95	87.4	4.68	83.6	4.06	89.8	5.07	94.8	2.56	91.4
incl. in a sea	8.68	69.3	4.14	73.9	3.92	86.7	4.97	92.9	2.47	88.2
coast	2.27	18.1	0.54	9.7	0.14	3.1	0.10	1.9	0.09	3.2
escapement to spawning	1.57	12.6	0.92	16.4	0.46	10.2	0.28	5.2	0.24	8.6
Eastern Kamchatka chum										
Stock	4.40		3.88		3.72		4.24		3.06	
Catch	2.65	60.2	2.66	68.6	3.24	87.1	3.97	93.6	2.33	76.1
incl. in a sea	0.73	16.6	1.40	36.1	2.80	75.3	3.70	87.2	0.83	27.1
coast	1.92	43.6	1.26	32.5	0.44	11.8	0.27	6.4	1.50	49.0
escapement to spawning	1.75	39.8	1.22	31.4	0.48	12.9	0.27	6.4	0.73	23.9
Amur chum										
Stock	11.09		11.42		9.08		9.08		5.09	
Catch	7.07	63.2	8.74	76.5	8.10	89.2	7.20	79.2	4.10	80.5
incl. in a sea	4.47	40.0	5.12	44.8	5.76	63.4	5.87	64.6	2.73	53.6
coast	2.60	23.2	3.62	31.7	2.34	25.8	1.33	14.6	1.37	26.9
escapement to spawning	4.12	36.8	2.68	23.5	0.98	10.8	1.88	20.7	0.99	19.5
Total number of Okhotsk continental coast, western and eastern Kamchatka, and Amur chum										
Stock	49.53		30.66		25.12		24.65		15.02	
Catch	35.50	71.7	23.00	75.0	20.46	81.4	20.91	84.8	11.40	75.9
incl. in a sea	22.67	45.8	14.96	48.8	16.50	65.7	18.93	76.8	8.10	53.9
coast	12.83	25.9	8.04	26.2	3.96	15.7	1.98	8.0	3.30	22.0
escapement to spawning	14.03	28.3	7.66	25.0	4.66	18.6	3.73	15.1	3.62	24.1

1959/60, it was impossible to compensate for the significant spawner deficiency during these years. This certainly affected the abundance of the newborn chum generation. In 1961 to 1965, its total stock was reduced to 30.66 million. In 1961 to 1965, a further decrease in the chum spawning run and its escapement rate was observed. During this period the chum escapement was 30 percent of optimum and 25 percent of average stock. The sea catch of the total chum stocks in question was 48.8 percent, though its volume was reduced by more than 7 million as compared to the previous years.

During the years 1966 to 1970 and 1970 to 1976, the chum stocks were decreased further due to a low reproduction rate in earlier years. From 1971 to 1976 chum stock was less than 50 percent of optimum. Nevertheless, in spite of the continuous chum stock decrease in 1966 to 1970 and 1971 to 1976, the sea catch even increased when compared to 1961 to 1965. During this period the high sea yield was 65.7 and 76.8 percent of total stock, respectively. This has caused a further decrease in spawning runs and hence a decrease of the coastal catch and escapement rate. In 1971 to 1976, the coastal catch was less than 10 percent of the level preceding the beginning of the period of the intensive high sea fishery (or 8 percent of that period stock) and escapement rate was less than 15 percent of the optimum.

The extremely low rate of reproduction in 1971 to 1976 was the basis of the unprecedented decrease of chum stocks (about 30 percent) for the years 1977 to 1979.

The years 1977 to 1979 are characterized by considerable changes in the exploitation system of salmon stocks because of the introduction of the 200-mile economical zones with the resultant limitations for the salmon high sea fishery.

Even though the chum sea catch for 1977 to 1979 decreased twice as much as in the previous years, and had an extremely low stock abundance, it has yet remained very high (approximately 54 percent) and total yield reached about 76 percent of the stock. In 1977 to 1979 chum reproduction was at the same catastrophically low level that resulted in the extremely low abundance of chum stock in the following years.

Analysis of the data on chum stock exploitation from 1955 to 1979 showed that during this entire period, heavy overfishing took place and the yield in these years was 72 to 85 percent (some stock yields reached 95 percent) of the stock. The main chum harvesting (over 50 percent and sometimes up to 60 to 77 percent) of the chum stock resulted in a lack of spawners in spawning grounds and a subsequent decrease in reproduction. The combined influence of these factors exceeded the level of species adaptation and anthropogenic factors played a leading part in this case. Table 2 shows how this factor influenced the reproduction with the data on chum escapement rate.

Table 2. Chum escapement rates (%).

Areas/Periods	1955-1960	1961-1965	1966-1970	1971-1976	1977-1979
Continental coast, Sea of Okhotsk	60.0	25.8	24.9	12.1	15.2
Western Kamchatka	26.2	15.3	7.7	4.7	4.0
Eastern Kamchatka	70.0	48.8	19.2	10.8	29.2
Amur River	74.9	48.7	17.8	34.2	18.0
Average by all regions	56.1	30.6	18.6	14.9	14.5

The optimum 100 percent escapement rate was that observed in 1945 to 1955.

Table 2 shows that during 25 years of highly developed sea fishery in all the main regions of chum reproduction, the steady reduction of the escapement and reproduction rates was observed (the latter reduced seven times).

Nevertheless, up to now the chum sea catch was very high. It is explained, particularly in the last decade, by large quantities of immature and young fish in sea catches. This has excluded the possibility of a reproduction increase and leads to the loss of the potential possibility of bioproduction increase as the average weight of the 3-year fish is half that of the 5-year fish. Last year the immature fish made up approximately 50 percent of the total annual chum catch on the high seas. Data on the age composition show that old age groups of fish in chum stocks have almost disappeared and do not reach 5- and especially 6-years of age. At the same time, the quantity of young fish (2-year +) has increased considerably in catches. It has been observed in the catches of both motherships and southern drifters. Similar changes of the age composition were observed in catches of south drifters.

If we take the chum age composition (Table 3) in catches of 1956 to 1960 for the natural

Table 3. Chum age composition in catches of motherships.

	Age (%)				
	1+	2+	3+	4+	5+
1956-1960		7.3	65.1	26.0	1.6
1961-1965		9.0	67.2	22.6	1.2
1966-1970		16.8	67.5	15.6	0.1
1971-1974	0.04	28.3	63.9	7.7	0.06
1975-1978	0.08	37.9	57.3	4.8	0.02

Area B: the Commander-Aleutian area. The northern perimeter borders the southern limit of area A; in the west its southern border stretches from 48°N to 165°E; in the east it stretches along 46°N to 165°E. In May up to 80 percent of the chum of the Okhotsk continental slope and 20 percent of chum of the western Kamchatka are caught in this area. In June the catch comprises 60 percent chum from the Okhotsk continental slope and 40 percent chum from western Kamchatka. In July and August it is 70 percent western Kamchatka chum, 20 percent chum from the Okhotsk continental slope and 10 percent of the Sakhalin, Kuril Island, Hokkaido and Honshu chum.

Area C: the southern area. In June and July the catch in this area was 60 percent Amur chum, 20 percent from the Okhotsk continental slope and 20 percent from the western Kamchatka. In July and August it consisted of 100 percent Amur chum.

The term "stock" adopted by the Soviet-Japanese Fisheries Commission for salmon means the sum of sea catch, coastal catch and escapement to spawning grounds; that is:

$N = C + R + P$, where

N = stock
C = sea catch
R = coastal catch
P = escapement to spawning grounds

In the first and following periods, the chum escapement to spawning grounds is calculated with low accuracy. It is based on the data on chum air and land observation stations in the rivers of different regions of the Far East.

Conventionally, we take 1955 to 1960 for the period of exploitation of optimum reproductive chum generation, with an annual stock average of about 500 million fish, with a 30 to 70 million fish fluctuation per year in the four main reproductive areas (Table 1): the Okhotsk continental slope, the western Kamchatka, the eastern Kamchatka and the Amur River. The annual catch until 1955 averaged 20 million fish or about 40 to 60 percent of the stock. About 20 million fish escaped to the spawning grounds for reproduction. We agree that the escapement of 60 percent of the chum stock to the spawning grounds is probably exaggerated and in some years the catch could be increased to 50 percent on the average.

The annual escapement of about 25 million fish to the spawning grounds, including escapement of 11 million to rivers of the Okhotsk continental slope, 6 million to the western Kamchatka rivers, 2.5 million to the eastern Kamchatka rivers and 5.5 million to the Amur River, is quite enough for the optimum level of natural chum reproduction in rivers of the areas in question.

The intensive high seas catch in 1955 to 1960 broke the optimum "yield-escapement" ratio and caused a significant decrease in escapement to spawning grounds and a heavy increase in fisheries. During this period the harvest was about 71.7 percent, including 45.8 percent of the sea catch and 25.9 percent of the coastal catch. The western Kamchatka chum was the most heavily fished. Its high sea catch averaged 69.3 percent and the coastal catch decreased to 18.1 percent. In 1955 to 1960 the total catch of the western Kamchatka chum averaged 87.4 percent.

In 1955 to 1960, the chum escapement to spawning grounds averaged about 14 million (28.3 percent of chum stock of this period) or 56 percent of the optimum spawning grounds capacity. As a result, the rate of reproduction was significantly reduced and chum escapement to western Kamchatka spawning grounds was only 12.6 percent of the total stock in this area or 26 percent of optimum capacity of the spawning grounds. The decrease of chum numbers in the spawning grounds in the Far East rivers, and especially those in western Kamchatka, is connected to the significant reduction of the chum run to the coastal area. As a result, the coastal catch was 36 percent lower than in previous years.

In 1961 to 1965, chum stocks were formed by 1955 to 1960 year classes. In spite of the favorable conditions for natural reproduction in the winter periods of 1955/56, 1956/57 and

ratio of age groups in mature parts of the population, then the increase of the young fish in the next years shows the obvious violation of the chum population structure; though one can surely say that in fact the age composition is quite different because of salmon fishing gear selectivity (at that time 55 mm mesh nets were used for these old age fish which were fished to a lesser extent than young ones).

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Temperature Regime in the Northern Part of the North Pacific in the Years 1962-1971

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Abstract

In order to know how to utilize the results of observations made by the fishery research ships authorized by the Fishery Agency of Japan during the years from 1962 to 1971, observed records were arranged for every statistical quadrant (one degree square), and data on water temperature were analyzed by the computer.

According to Table 1, shown as an example of the results, an area covered by the temperature quadrants over 5°C at zero m layer is most extensive in July and narrowest in May. The author should report some other results on the structure of temperature fields by applying colored grids.

Table 1. Number of the temperature quadrant (= area of a temperature range at the layer 0m, 10m and 50m in May, June, July and August for ten years 1962-1971.

Month	Temperature range / Depth (m)	Over 5°C	4.00-4.99°C	3.00-3.99°C	2.00-2.99°C	1.00-1.99°C	0.00-0.99°C	Below 0°C
May	0m	25	115	44	1	0	0	0
	10m	14	95	42	9	0	0	0
	50m	1	34	102	26	8	1	0
June	0m	307	20	1	1	0	0	0
	10m	246	61	8	0	0	1	0
	50m	21	83	121	66	26	1	1
July	0m	332	0	0	0	0	0	0
	10m	304	1	1	0	0	0	0
	50m	73	76	66	76	12	2	1
August	0m	121	0	0	0	0	0	0
	10m	73	0	0	0	0	0	0
	50m	21	9	14	15	6	1	1

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Some Hatchery Strategies for Reducing Predation upon Juvenile Chum Salmon (*Oncorhynchus keta*) in Freshwater

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Abstract

In the pilot phase of a multi-year study, the effectiveness of some artificial propagation strategies in reducing freshwater predation upon juvenile chum salmon (*Oncorhynchus keta*) was experimentally tested. Studies were conducted by releasing marked and unmarked groups of fed and unfed chum juveniles into a small stream and recapturing them at a downstream weir. The freshwater survival of these groups was measured as a function of group size at release, time of release, release location, fish size at release, and day versus night hatchery feeding.

In the experiment studying the effects of releasing different numbers of juvenile chum, freshwater survival varied from 40 percent when 517 fry were released to 92 percent when over 50,000 were released. In trials when the time of release was varied, survival was 73 percent for unfed fry released approximately 1.5 hours before sunset as opposed to 80 percent when released approximately 1.5 hours after sunset; survival was also estimated to be greater for both diurnally and nocturnally fed chum that were released at night (2345 hr) than during the day (1100 hr). When studying the effects of downstream migration distance, 48 percent of the fry survived when released 10.0 km above the weir as compared to 74 percent when released 2.3 km above the weir. In an experiment comparing the survival of fed and unfed chum, 46 percent of a group of unfed fry ($\bar{x}$ = 39.2 mm fork length) survived as compared to 85 percent of a group of fed chum ($\bar{x}$ = 55.7 mm fork length). Nocturnal feeding appeared to result in a slight survival advantage relative to diurnal feeding when chum were released in the day.

The freshwater survival of juvenile chum salmon can be increased with hatchery practices that: mimic natural behavior patterns (e.g., by releasing fish at night), minimize downstream travelling distance, feed fish in hatcheries at night, release bigger chum, and release larger numbers of chum.

Introduction

It has long been recognized that predation can be an important cause of mortality of juvenile Pacific salmon (*Oncorhynchus* spp.), particularly in freshwater (Abramov, 1953; Bakshtanskii, 1970; Eggers, 1978; Foerster and Ricker, 1941; Hunter, 1959). Several studies have estimated that up to 85 percent of juvenile salmon outmigrants can be removed by freshwater predators (Hunter, 1959; Neave, 1953; Semko, 1954). Because of the potential magnitude of predation mortality on juvenile salmon and its significance in salmon population dynamics, we initiated a research program in spring 1980 at the University of Washington's Fisheries Research Station at Big Beef Creek, Washington, to: 1) investigate freshwater predation of juvenile chum salmon (*O. keta*); and 2) study means of minimizing freshwater losses of chum to predators. Chum were chosen for study because this species has been selected recently for massive enhancement efforts in Washington state. Experiments conducted during the pilot phase of the program are described in this paper. Our first-year studies sought to experimentally test the effectiveness of several hatchery practices

in reducing freshwater losses of juvenile chum to predators following release. While we expect techniques that increase the freshwater survival of salmon to translate into increased adult survival, only by studies spanning the entire life cycle can this be confirmed.

Studies were conducted by measuring the survival and describing the migratory behavior of groups of fed and unfed juvenile chum salmon between the time of their release into Big Beef Creek and subsequent arrival at a weir situated at the stream's mouth. Our experiments examined the effects of different release numbers, day versus night hatchery feeding, time of release, migration distance, and chum size at release.

Experimental Design

Study Stream

Big Beef Creek is a small, lowland stream draining into the eastern side of Hood Canal, a fjord-like extension of Puget Sound with spring stream flows typically ranging from 0.4 to 2.5 m^3 sec^{-1}. Originating in marshes and beaver ponds, the stream courses 8.0 km into a 198-hectare reservoir before descending 10.0 km through a steep gully into Hood Canal. A permanent upstream-downstream fish trap and weir, which can trap all ascending adult salmon or descending juveniles, is located at the mouth of the stream. The principal, potential predators of juvenile chum in this stream are coho salmon (*O. kisutch*), cutthroat trout (*Salmo clarki*), rainbow (steelhead) trout (*S. gairdneri*), prickly sculpin (*Cottus asper*), and coastrange sculpin (*C. aleuticus*) (Fresh, et al., in prep.); small mammals and birds such as otters and kingfishers may also eat juvenile chum.

Fish Handling Procedures

Big Beef Creek has early, middle, and late timed chum runs from which the fry normally migrate seaward from late January through June. Upstream escapements of adult chum were controlled to create a period of time when no wild fry were emigrating that was also prior to the peak emigration in May and June of three major chum predators: coho salmon, steelhead trout, and cutthroat trout. Accordingly, experiments were conducted in March and early April 1980. Egg takes were manipulated to ensure that experiments were completed during this period.

Experiments were carried out using only the progeny of the native chum salmon stock of Big Beef Creek. All eggs were incubated in 10°C well water in Heath incubator trays with 1.5 inch bioring substrate until the K_D (in a condition factor after Bams, 1967 and 1970) of the fry was between 1.80 and 1.89; at this time, they were judged ready for release or transfer to tanks for feeding studies.

Most groups of chum were spray-marked with different colors of fluorescent pigment prior to release, using procedures described by Whitmus and Olsen (1979), in order to distinguish experimental groups. Groups of unmarked (i.e., not subjected to the complete marking procedure) and marked chum were released simultaneously on several occasions to evaluate whether the marking procedure may have imposed a stress that increased vulnerability to predators. Unmarked groups and the marked groups being tested were treated identically, with the exception of the complete marking procedure.

Handling procedures were standardized for all experimental release groups. Marked (and unmarked) groups were held for approximately 48 hours after marking to evaluate mortality; the majority of chum injured by the marking procedure died within 24 hours after marking. Most survivors of each group were then transported to the stream where they were acclimated for 1.5 hours and released. The remainder (100 to 600 fish per group) of each group (i.e., those fish not released) was retained in order to measure mark retention and further mortality. Mark retention was determined for each group at release and after the majority of the group had reached the weir (usually within 24 hours after release) by examining fish from

the group that was retained; there was no significant difference between pigment retention at release and 12 to 24 hours later (Mann-Whitney U-test, $p > 0.05$).

Weir Monitoring and Catch Processing

Fish catches at the weir were enumerated every 0.5 to 2.0 hours throughout the study. All fish were identified and counted (individually or, when catches exceeded 5,000 fish per night, gravimetrically) and a portion of the catch of each species measured for length (fork length [FL] to the nearest 1.0 mm for all salmon) and weighed (nearest 0.01 g). All chum or a random subsample were examined for the presence of fluorescent pigment.

Calculation of Juvenile Chum Survival

Freshwater survival was determined by using only chum with pigment and was calculated as the ratio of the number of chum with pigment released upstream. Survival estimates were for the 48-hour period after release, as results showed most (> 97 percent) outmigrants from experimental releases reached the weir within this period. We assumed chum not recaptured were mortalities resulting from predation. Marked chum were found in the stomachs of predators caught at the weir and by electroshocking, and predators were observed actively feeding on chum immediately after chum were released (Fresh, et al., in prep.). Studies in other similar streams (e.g., Hunter, 1959) with comparable predator species also attribute most losses of juvenile salmon to predation. In future studies, we will seek to more directly relate observed losses of juvenile chum to freshwater predator populations and consumption rates.

Experimental Releases

Effect of the Numbers of Fry Released

Current hatchery practices regarding the number of salmon per release vary considerably, ranging from large, single releases to volitional releases where smaller numbers are allowed to migrate at intervals over several weeks. However, the survival of a group of salmon can perhaps directly relate to the numbers released (i.e., density) (Peterman and Gatto, 1978). To determine the relationship between survival and numbers of chum released, five groups of unfed fry, ranging in number from 517 to 50,155 fish, were released at river kilometer (RK) 2.3 within an 11-day period (Table 1).

Effect of Time of Release

Since juvenile salmon predators are primarily visual feeders (Patten, 1971; Eggers, 1978) and chum fry naturally migrate mostly at night (Hunter, 1959; Kobayashi, 1958 and 1964; Neave, 1955), survival should be greater for chum released at night than during the day. To test this, unfed and fed chum were released at RK 2.3 at times between 1100 and 2345 hours (Table 1).

Effect of Migration Distance

Unfed fry were released approximately 1.5 hours before sunset at RK 2.3 on 18 March and at RK 10.0 on 23 March to determine how migration distance, and therefore exposure to predators, affects survival (Table 1).

Effect of Nocturnal Versus Diurnal Hatchery Feeding

Several studies suggested that diurnal feeding (i.e., rearing) in hatcheries may condition salmon fry to behavior patterns that increase their vulnerability to predation (Volovik and Gritsenko, 1970) and reduce their growth (Kobayashi, 1960), as compared with feeding at night. To test the effects of nocturnal versus diurnal hatchery feeding on chum growth and vulnerability to predation, two groups of chum were reared in identical tanks on Oregon Moist Pellets and fed at 5 percent body weight per day. With automatic feeders, one group

Table 1 Summary of the characteristics of each experimental group of chum salmon juveniles into Big Beef Creek, March-April 1980.

Group and experiment[1]	Date released	Time released (PST)[2]	Where released (river km)	Number released	Feeding regime[3]	Mark[4]	Pigment retention (%)[5]	Fork length at release (mm)		Weight at release (g)		Conditior factor, K_D[7]	
								Mean	S.D.[6]	Mean	S.D.	Mean	S.D.
Unfed													
ERT, EM	3/16	1947	2.3	904	—	B	64.3	39.7	1.4	0.38	0.05	1.83	0.06
ERT, EM	3/16	1947	2.3	1,223	—	U	—	—	—	—	—	—	—
ERT, EM, ED	3/18	1700	2.3	994	—	R	69.6	39.7	1.3	0.37	0.05	1.81	0.04
ERT, EM, ED	3/18	1700	2.3	1,377	—	U	—	39.8	1.5	0.38	0.05	1.80	0.04
ENR	3/20	1705	2.3	4,727	—	O	71.5	39.4	1.3	0.36	0.07	1.80	0.09
ENR	3/23	1710	2.3	15,186	—	R	62.6	40.2	1.3	0.39	0.04	1.82	0.04
ENR	3/26	1705	2.3	50,155	—	O	52.7	39.1	1.3	0.36	0.04	1.82	0.03
ENR	3/30	1706	2.3	517	—	R	65.9	39.0	0.9	0.36	0.03	1.82	0.03
ENR	3/31	1708	2.3	1,206	—	O	68.4	38.8	0.9	0.34	0.03	1.89	0.04
ED	3/23	1710	10.0	2,115	—	G	58.1	40.0	1.2	0.40	0.03	1.84	0.06
EFS	4/13	1700	2.3	994	—	O	51.8	39.2	0.7	0.37	0.02	1.83	0.03
Fed													
DN, ERT, EM	4/6	1100	2.3	551	N	O	89.4	50.7	2.4	0.89	0.12	—	—
DN, ERT, EM	4/6	1100	2.3	548	D	R	89.4	53.2	2.7	1.08	0.15	—	—
DN, ERT, EM	4/6	1100	2.3	605	D	U	—	52.3	2.8	1.00	0.17	—	—
DN, ERT, EM	4/9	2345	2.3	793	N	O	73.6	52.7	3.0	1.09	0.21	—	—
DN, ERT, EM	4/9	2345	2.3	750	D	R	72.0	55.3	3.0	1.24	0.21	—	—
DN, ERT, EM	4/9	2345	2.3	817	N	U	—	53.3	2.8	1.10	0.14	—	—
EFS	4/11	1703	2.3	1,251	D	R	71.5	55.4	3.1	1.17	0.19	—	—

[1]ERT = Effect of Release Time; EM = Effect of Marking; ED = Effect of Downstream Migration Distance; ENR = Effect of the Number of Fry Released; EFS = Effect of Fry Size at Release; DN = Diurnal-Nocturnal Hatchery Feeding.

[2]Pacific Standard Time.

[3]D = Diurnal; N = Nocturnal.

[4]U = Unmarked; R = Red; O = Orange; B = Blue; G = Green.

[5]To determine the number of chum released with pigment, multiply the mark retention by the number released.

[6]Standard deviation.

[7]K_D = Condition Factor of Bams (1967, 1970).

was fed during the day and the other was fed at night. The biomass of chum in each tank was equalized by twice weekly adjustments of fish. Portions of both groups were released concomitantly at 1100 hours on 6 April and 2345 hours on 9 April (Table 1) after one group had reached an average weight of approximately 1.1 g, the approximate size at which chum are released from Washington Department of Fisheries (WDF) hatcheries (Antipa, 1980).

Effect of Chum Size at Release

That size is an important factor affecting the survival of juvenile salmon has been demonstrated in a number of studies (e.g., Bams, 1967; Beall, 1972). In Japan, the recent increase (since 1970) in chum salmon populations has been largely attributed to rearing chum to a larger size before release (Kobayashi, 1976). The WDF also regularly rears chum prior to their release (Antipa, 1980). To determine whether releasing larger chum improves survival during the freshwater outmigration, similar numbers of fed ($\bar{x}$ = 55.7 mm FL) and unfed chum ($\bar{x}$ = 39.2 mm FL) were released at RK 2.3, approximately 1.75 hours before sunset (Table 1).

Results and Discussion

Chum Behavior

To determine how long it would take the majority of a group of chum to reach the weir after release, and thus define the rate at which experimental releases could be made, one trial group of fed and one group of unfed chum juveniles were released at RK 2.3. Plotting the cumulative percentage of the number of chum arriving at the weir to the number released over time for the two releases (Figure 1) indicated that most chum left the stream rapidly (within 48 hours) after release.

Over 99 percent of all chum captured entered weir traps at night, regardless of size at release, release time, or distance traveled. Studies of wild chum fry behavior also show that most fry movement occurs at night (Hunter, 1959; Kobayashi, 1958 and 1964; Neave, 1955), apparently because juvenile chum are especially sensitive to light (Neave, 1955). Even bright moonlight can inhibit migratory activity (Kobayashi, 1964). However, as McDonald (1960)

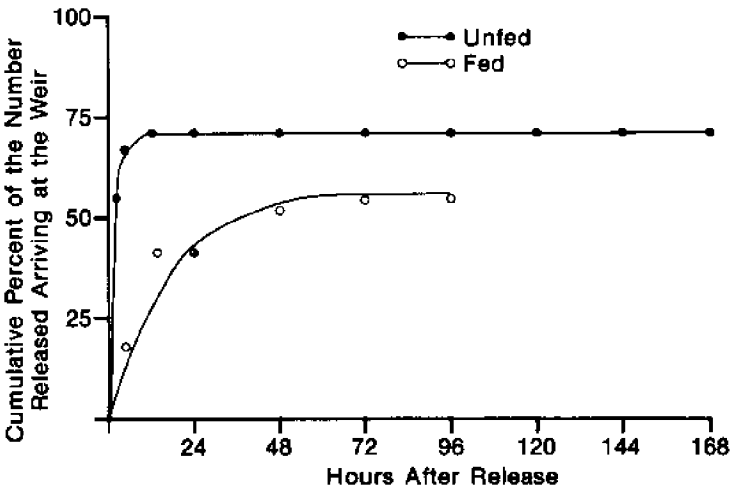

Figure 1. Cumulative percentage of the number of chum arriving at the weir to the total released over time for one test release of unfed and one test release of fed chum juveniles. Both releases took place from RK 2.3; 1,000 unfed chum fry were released on 8 March and 287 fed chum were released on 2 April. (Note: because the number of fish released in each group was different, it is not possible to compare the survival of the two groups.)

and Bakshtanskii (1970) indicate for pink salmon (*O. gorbuscha*) fry, the tendency for chum to migrate during the day probably increases when downstream travel distance increases.

Effect of Marking

Groups held after marking had significantly more mortalities after 48 hours than did groups that were otherwise similarly treated but not subjected to the complete marking procedure (Mann-Whitney U-Test, $p < 0.01$).

Expected recoveries of marked chum were estimated by assuming that for chum subjected to the marking procedure, the presence or absence of pigment on chum did not affect vulnerability to predators. For each trial, the rate of the number of chum released with pigment to the total released was multiplied by the recoveries at the weir within the 48-hour period after release. We believed mixing of unmarked fish from successive releases would be minimal because chum rapidly emigrated. For the two trials with unfed fry, the observed recoveries of marked chum were less than expected by 10.9 percent and 0.2 percent (Table 2), suggesting the marking procedure did not substantially increase vulnerability to predation.

For fed chum trials, the observed recoveries were fewer than expected by 2.3 percent in the test with diurnally fed chum and greater than expected by 8.7 percent in the test with nocturnally fed chum (Table 2), again suggesting the marking procedure did not substantially increase vulnerability to predation.

Table 2. Results of experiments to study whether spray-marking juvenile chum salmon increases their vulnerability to freshwater predators after release into Big Beef Creek, Washington, March-April, 1980.

Group	Date and time released	Estimated number of chum released with pigment	Expected marked recoveries after 48 hr	Observed marked recoveries after 48 hr	% Deviation from the expected[1]
Unfed	3/16—1947	580	521	464	-10.9
Unfed	3/18—1700	693	508	507	-0.2
Fed (diurnally fed)	4/6 —1100	490	360	352	-2.3
Fed (nocturnally fed)	4/9 —2345	583	507	551	+8.7

[1]Expected > observed = -.
Expected < observed = +.

Effect of Numbers of Fry Released

Freshwater survival increased non-linearly with numbers of unfed fry released, ranging from 40.3 percent when 517 fry were released, to 91.5 percent when 50,155 were released (Figure 2). The percentage gain in survival was substantially greater for low numbers released than for high. The relationship of mortality to the numbers released (density) indicated that mortality was inversely density-dependent (depensatory [Neave, 1953]). The migration behavior of the five groups was generally similar, except that a greater percentage (approximately 8 percent versus approximately 1 percent) of the smaller groups (< 1,300 released) emigrated after the first 24 hours.

Effect of Time of Release

For the trial with unfed fry, survival was 80.0 percent when released 1.5 hours after sunset and 73.3 percent when released approximately 1.5 hours before sunset. For diurnally fed chum, survival was 93.7 percent when they were released at 2345 hours and 71.8 percent when released at 1100 hours. For nocturnally fed fish survival was 94.5 percent versus 80.7 percent for release at 2345 hours and 1100 hours, respectively. However, the different composition of the two fed trials may have biased the survival estimates. The release at 1100

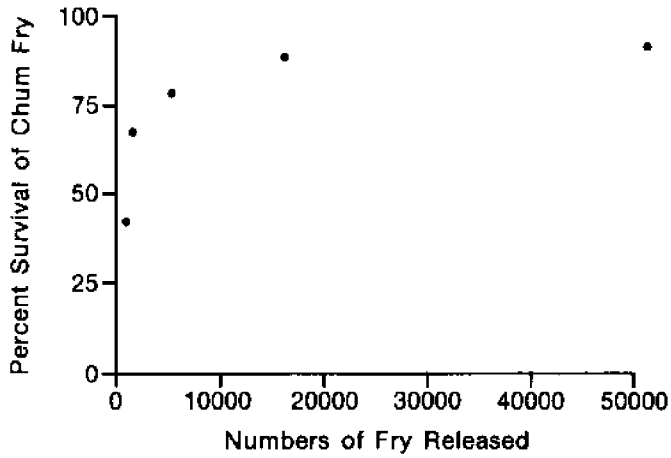

Figure 2. Freshwater survival of unfed chum fry released into Big Beef Creek as a function of numbers released. All releases took place from RK 2.3, 1.5 hours before sunset, 20-31 March 1980.

hours had twice as many diurnally as nocturnally fed fish while the night release was opposite (Table 1); as a result, some of the observed difference in survival may be a function of the differing densities of chum from each feeding strategy in the two releases. The difference in survival between the day and night releases of both rearing strategies was substantial enough that we feel the direction of the differences would remain constant, only the absolute value would change.

Effect of Migration Distance

Survival was estimated to be 73.7 percent for unfed fry released at RK 2.3 and 48.2 percent for release at RK 10.0, showing that increased exposure to predators decreases survival. Our results are consistent with those of Cameron (1958) and Foerster (1968) for pink and sockeye salmon (O. *nerka*) fry, respectively.

The first migrants arrived within 0.5 hours and 7.5 hours from release at RK 2.3 and RK 10.0, respectively. Increasing the migration distance did not increase the proportion of fish recovered in the day since less than 0.5 percent of fry from release at both locations were caught at the weir during the day. Greater distances than we tested are probably needed to increase the degree of daytime movement. McDonald (1960) found that, in general, the degree of daytime movement of pink salmon fry increased with increasing distance fry might have traveled. At one site where the spawning grounds extended 88 km above the trapping site, McDonald captured 65 percent of the pink fry in the day, whereas at another site where the spawning grounds extended 6 km above the site, no fry were captured during the day.

Effect of Nocturnal Versus Diurnal Hatchery Feeding

At release, chum fed in hatchery tanks during daylight hours averaged nearly 3.0 mm longer in fork length than chum fed at night. While the difference in size may reflect a better growth potential of the diurnally fed fish, it may also be a result of: 1) insufficient illumination for the nocturnal group; or 2) inconsistent feeding of the nocturnal group. When daytime and nighttime fed fish were simultaneously released in the day, the freshwater survival of chum fed at night was greater than for chum fed in the day (80.7 percent versus 71.8 percent) (Table 3). The difference in survival was much less (Survival was 93.7 percent for the daytime fed group as compared to 94.5 percent for the night fed group.) when the two groups were released simultaneously at night. Nocturnally fed fish may have had better survival when released during the day because they were conditioned to relatively greater inactivity (e.g., hiding) during daylight hours when visual predators would be most effective

Table 3. Comparison of the freshwater survival in Big Beef Creek of chum salmon juveniles fed during the day and at night, April 1980.

Rearing schedule	Date released	Time released	Marked (M) or unmarked (U)	Number released	Percent survival
Diurnal	4/6	1100	M	551	71.8
Nocturnal	4/6	1100	M	548	80.7
Diurnal	4/6	1100	U	605	—
Total released				1,704	
Nocturnal	4/9	2345	M	793	94.5
Diurnal	4/9	2345	M	750	93.7
Nocturnal	4/9	2345	U	817	—
Total released				2,360	

(Volovik and Gritksenko, 1970). On the other hand, diurnally fed chum may have been conditioned to greater activity in the day, and thus more subject to predators. However, potential biases (i.e., the different ratios of diurnally and nocturnally fed fish that were released each time) that have been previously discussed may have affected survival estimates.

Effect of Fish Size at Release

Survival of unfed chum fry was 46.2 percent as compared to 84.7 percent for the larger, fed fish. Other studies have pointed out the importance of size of salmon to survival. Bams (1967) found that the performance of sockeye fry in predation vulnerability and stamina tests was directly related to size. Hiyama, et al. (1972) released two groups of different sized chum into the Otsuchi River of Japan and estimated that 90 to 100 percent of the larger chum ($\bar{x}$ = 50 mm FL) as compared with 60 to 64 percent of the smaller chum ($\bar{x}$ = 36 mm FL) reached a downstream fish trap within the three-day test period. Beall (1972) found that smaller chum were selectively preyed on by coho but not by cottids.

Hatchery Practice Implications

In this preliminary phase of our studies we have examined how the freshwater losses of juvenile salmon, primarily due to predation, can vary with artificial propagation strategies. While predation has manifold and complex attributes that will be unique to each system, our results suggest hatchery practices that can reduce freshwater predation on juvenile chum salmon. These practices should be applicable not only to chum salmon but also to other salmon species because similar basic principles are involved.

Results of our time of release experiments suggest that chum should be released at night, since during darkness chum have the best chance of avoiding visual predators. This practice mimics a natural behavior pattern of chum and its success suggests that other practices that mimic natural behavior patterns will increase freshwater survival. This conclusion is supported by other investigators who have found that survival can be increased by: incubating in gravel (e.g., Bams, 1972), avoiding light during incubation (e.g., Smith, 1916), releasing fry according to the natural timing of runs (Kobayashi, 1976), and releasing fry during advantageous environmental conditions (e.g., high river velocity and turbidity) (Ginetz and Larkin, 1976).

Our initial results indicated that feeding chum in hatcheries at night can also increase freshwater survival. However, problems with our experimental design necessitate further studies to determine differences in growth patterns and predator vulnerability.

Releasing larger numbers of chum at one time should also result in increased survival, which suggests that such practices as volitional releases may result in decreased survival. Volitional releases may also be counterproductive in that they probably attract predators in unnecessarily large numbers and hold them in the area of release. It is not possible at this

time to determine whether optimum release numbers can be universally predicted because predator populations and environmental conditions may be too variable year-to-year and stream-to-stream. However, results of enhancement efforts may not be as great as expected until we can determine at what release densities predators are swamped (Peterman and Gatto, 1978). Release densities should be considered in the context of food limitations in estuarine and marine rearing areas as well because extremely large releases may exceed carrying capacities and result in lower survivals (Simenstad, et al., in prep.).

Releasing larger chum, as is currently widely practiced, will also increase freshwater survival. Emphasis should be placed on establishing rearing regimes whereby releases will coincide with natural emigrations and maximum marine food supplies (Kobayashi, 1976). Releases should occur before marine water temperatures reach 15°C since growth, appetite, food conversion efficiency, and digestion rates may decline at temperatures above 15°C (Brett and Higgs, 1970).

Releases of juvenile chum should occur as close to estuaries as possible, since the further chum must migrate, the greater their exposure to predators and the higher the losses. New culturing facilities should be sited as close to estuaries as possible to minimize the distance chum have to travel in freshwater.

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Some Effects of the Marine Environment on Age at Maturity and Growth of Chum Salmon in Prince William Sound, Alaska

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Abstract

Influence of the marine environment on age and size at maturity, early marine growth, and abundance of chum salmon, *Oncorhynchus keta*, from Olsen Creek in Prince William Sound, Alaska, was studied during the years 1959 to 1978 (Helle, 1979).

Age composition of the spawners returning to Olsen Creek varied from year to year; but they were predominately 3-, 4-, and 5-year fish. Some 6-year fish returned between 1968 and 1975, but this age group usually did not represent more than 1 percent of the returns. Mean age composition for the brood years 1956 to 1972 for males was 15, 66, and 19 percent for 3-, 4-, and 5-year fish, respectively. Mean age composition for females of the same broods showed higher percentages of older fish: 9, 67, and 23 percent for 3-, 4-, and 5-year fish, respectively.

Interseasonally, mean age at maturity increased as number of fish in a brood increased. Intraseasonally, age of new chum salmon spawners at Olsen Creek decreased as the season progressed. Mean size of older spawners was significantly larger than the mean size of younger spawners, but the ranges in size of the three age groups overlap each other so size is not a good criterion for estimating age of chum salmon.

Numbers of circuli and distances between annuli on adult scales were used to estimate growth of chum salmon during their first two years at sea. Growth during the first year at sea was related to sea-surface temperatures and marine weather parameters in Prince William Sound and in the northern Gulf of Alaska. Growth during the first year at sea was not significantly related to age at maturity; however, growth during the second year at sea was negatively related to age at maturity.

Size at maturity was related to sea-surface temperatures and marine weather parameters in the northern Gulf of Alaska and Prince William Sound during the year of return. Fluctuations in size at maturity were more similar between fish from different broods returning during the same year than between fish from the same broods maturing at different ages. A highly significant relationship was found between survival of progeny and mean size of the parents.

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Fluctuations in Abundance of Sockeye Salmon of the Ozernaya River Stock

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Sockeye stock spawning in the basin of the Ozernaya River and primarily in Kurilskoye Lake represents one of the largest local stocks of *Oncorhynchus nerka* Walb on the Asian coast of the Pacific.

The state of Ozernaya sockeye stock has been studied since 1940. There are studies on fluctuations in sockeye abundance (Krogius and Krokhin, 1956; Egorova, et al., 1961), methods of distinguishing sea catches (Krogius, 1967) and influence of the harvesting on abundance (Krogius, 1961; Menshutkin and Kislyakov, 1968; Seliphonov, 1975). Most of these were carried out when the stock abundance was sufficiently high to provide a balance between harvesting and escapement.

The task of this work is to assess the present state of Ozernaya sockeye stocks and investigate the influence of the high sea fisheries on fluctuations in its abundance.

Materials and Methods

Data on ichthyological samples were taken in the areas of the Ozernaya River and Kurilskoye Lake during 1940 to 1979, and from reports of the Soviet-Japanese Fisheries Commission as well.

The number of spawners was determined by their total estimation on the counting fence of the Ozernaya River. Fish quantity, size, and sex structures in the coastal catches were calculated by the medium weight of sockeye determined during biological analysis. Ozernaya sockeye has been distinguished from the sea catches by the methods of F. V. Krogius (1967) and modified by M. M. Seliphonov (1975) which provides an analysis of sockeye distribution by the areas and time as follows: a) age structure of the stock; b) scale structure; and c) run terms and the state of the reproductive organ maturity.

The Results of the Investigation

Ozernaya sockeye stocks are estimated by three indices: escapement rate of spawners and two volumes of coastal and sea catches.

The period from 1940 to 1975, when abundance of the whole mature part of the stock had been determined by the degree of the fisheries' influence and the state of Ozernaya sockeye abundance, may be divided into five stages (Table 1). The first stage covers 1940 to 1944 with sockeye data provided by the Soviet and Japanese fisheries in the coastal waters of Kamchatka. The second stage was 1945 to 1952 when sockeye were harvested only by the Soviet coastal fisheries. The third stage (1953 to 1963) was the peak of the sea fisheries with

Table 1. Mature part of Ozernaya sockeye stock.

Periods	Years of spawning	Numbers of spawners	Catches in the sea	Escapement in the river	Catches in Japan	Mature part of the stock	Intensity of the fisheries
				in thousands			
1	1940-1944	1140	962	2102	3274	5376	4236
2	1945-1952	2469	2779	5248	96	5344	2875
3	1953-1963	1143	1186	2329	4221	6550	5407
4	1964-1969	670	471	1141	2770	3911	3241
5	1970-1975	438	221	659	1370	2029	1591
6	1976-1979	987	168	1159	—	—	—
				percents from the mature part of the stock			
1	1940-1944	21.21	17.89	39.10	60.90	100.0	78.79
2	1945-1952	46.20	52.00	98.20	1.80	100.0	53.80
3	1953-1963	17.45	18.11	35.56	64.44	100.0	82.55
4	1964-1969	17.13	12.04	29.17	70.83	100.0	82.87
5	1970-1975	21.59	10.89	32.48	67.52	100.0	78.41

intensive fishing of Ozernaya sockeye in the high seas. Since 1964, however, the fishing intensity has remained constant, but the abundance of the mature portion of the stock has been declining. Since 1970, the total abundance of Ozernaya sockeye has stabilized at an extremely low level of 1.5 to 2.3 million fish. The intensity of the fisheries decreased somewhat mainly in the coastal fishery from 82.87 to 78.41 percent, but was still at a very high level.

In the fifth stage, the number of spawners decreased 2.6 times as much as the 1953 to 1964 period and 5.6 times the period of 1945 to 1952. From 1976 to 1979, an apparent increase of sockeye escapement was observed.

In these years, the harvest of Ozernaya sockeye was fixed in the range of 200,000 fish. In 1970 to 1975, a considerable reduction of sockeye catch by the coastal fisheries was observed. In this period, the catch in the river decreased 12.6 and 5.4 times, respectively, as compared to the second and the third periods. Along with the decrease of fish caught by the coastal fisheries in the second period and later on, the share of the coastal catches in the total abundance of the mature portion of the stock also declined.

The high seas fisheries have been developed since 1952. In 1955 to 1957, sockeye catches reached a maximum with an annual catch of 10 to 20 million sockeye. Ozernaya sockeye ranged from 35.6 to 51.6 percent of the total catch of sockeye. Later on these percentages decreased along with the decline of the total catch. In 1970 to 1975, the harvest was from 16.3 to 31.9 percent (average of 20.6 percent). The absolute number of Ozernaya sockeye caught on the high seas in 1974 totaled 0.9 million. In 1957, this catch did not exceed 7.1 million. From the start of the fisheries up to the fifth period, the total catch of Ozernaya sockeye in the high seas has decreased by 3.1 times. However, the intensity of the high seas fisheries remained high. The intensive harvesting of the mature portion of the stock (on the order of 80 percent) is probably the main reason for the reduction in the abundance of Ozernaya sockeye stock (Table 2).

Another important reason for the decline of stocks was a considerable catch of immature fish in the Northwestern Pacific. Before the fishery was started in the area south of 48°N (1967), the harvest of Ozernaya sockeye was about 20 percent (average of 15.54 percent) of the total catch. Later on, as the average yield of the mature fish dropped, the number of immature sockeye in the catch increased to about 37.42 percent. As shown earlier, the harvest of the immature fish is very unreasonable (Seliphonov, 1978) since it leads to the loss of biomass of the caught fish on the one hand and reduces recruitment on the other hand. It has been determined that not more than 10 percent of the immature fish may be caught without any detriment to reproduction. For this reason, it is necessary to completely stop harvesting sockeye in the area south of 48°N.

Table 2. Catches of Ozernaya sockeye in the sea (in thousands).

Year	Mature	Immature	Numbers of immature, %
1962	4426	615	12.20
1963	3529	726	17.06
1964	1370	334	19.60
1965	2034	496	19.60
1966	2954	463	13.55
1967	4066	1610	28.36
1968	3386	1329	28.19
1969	2812	554	16.46
1970	1741	1404	44.64
1971	1472	749	33.72
1972	1351	1312	49.27
1973	1129	2087	64.89
1974	887	946	51.61
1975	1639	1059	39.25
1962-1966	2863	527	15.54
1967-1975	2054	1228	37.42

Abundance reduction of Ozernaya sockeye population could not help but influence the stock structure and primarily its age composition in 1940 to 1963 when Ozernaya sockeye stock were at a satisfactory level. In two basic age groups, the mature portion of the stock dominated the 5_{2+} aged fish (Table 3). Later on following the reduction of the total abundance, sockeye in the 4_{2+} age group prevailed in separate years and annually during 1970 to 1975, excluding 1974. At the same time, considerable declination of Ozernaya sockeye stock was followed by the reduction in average age of the population. Since 1953, the decrease in fish quantity of two main age groups, 4_{2+} and 5_{2+}, has taken place in the mature portion of the stock (Table 3), with the 5_{2+} declining more rapidly than the 4_{2+} increase in the mid-70s. The abundance of the old age groups, 6_{2+} and 6_{3+}, is also reduced. On the other hand, the increase of mature male (3_{2+}) abundance is also marked. The reduction in average age of the population following Ozernaya sockeye reduction in abundance, has also been observed earlier (Egorova, et al., 1961). Birman (1951), on the basis of the Amur River fall chum studies, showed that the increase of fish abundance results in the reduction of growth rate and increase of fish quantity of all age groups. The reduction in average age of Ozernaya sockeye population is caused probably by the following factors: abundance reduction leads

Table 3. Age structure of the mature part of Ozernaya sockeye stock.

Periods	Age					
	3_{2+}	4_{2+}	5_{2+}	6_{2+}	5_{3+}	6_{3+}
	in thousands					
1940-1944	—	1388	3988	—	—	—
1945-1952	13	1324	3092	58	569	288
1953-1963	37	2039	3222	89	632	530
1964-1969	36	1500	1830	27	318	201
1970-1975	64	990	667	7	220	81
1940-1975	31	1525	2642	46	409	273
	Percents					
1940-1944	—	25.82	74.18	—	—	—
1945-1952	0.23	24.78	57.86	1.09	10.65	5.39
1953-1963	0.56	31.14	49.20	1.36	9.65	8.09
1964-1969	0.92	38.34	46.78	0.69	8.13	5.14
1970-1975	3.16	48.79	32.87	0.35	10.84	3.99
1940-1975	0.63	30.96	53.63	0.94	8.30	5.54

to rapid fish maturity in the sea; and during the whole sockeye lifespan, the dominant age group, 5_{2+}, is harvested twice. That is why the fisheries pressure on this age group is considerably higher than on the other groups.

The annual influence of the fisheries has resulted in changes of the intrapopulation structure of the stock. In addition to the lack of spawners, these changes have negatively influenced the state of Ozernaya sockeye stock.

F. V. Krogius (1961) has pointed out that fishing pressure affects the separate sockeye stocks of the Asian and American coasts. Intensive harvesting at sea leads to a reduction of all stocks in spite of favorable reproduction conditions.

Different behavior variants of Ozernaya sockeye stock under varying fishing conditions were studied by V. V. Menshutkin and Yu. Ya. Kislyakov. They showed that with an escapement of 1 million spawners, the rate of high sea exploitation must not exceed 25 to 35 percent of the mortality rates. More intensive sea fishing might result in the destruction of the stock.

In 1978/1979, the escapement rate of spawners was 1.45 and 1.35 million. However, this increase is not related to the enhancement of stock abundance, but is related to the reduction of the high sea fisheries. In these years, according to the estimated catch of Ozernaya, sockeye in the sea was about 30 percent of the total abundance of the immature portion of the stock. A further reduction of harvesting would allow hope for the restoration of Ozernaya sockeye stock.

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Section II
Advances in Artificial Propagation of Aquatic Animals

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The Role of Salmon Production and the Perspectives of its Development in the Sakhalin Region

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At present there are 23 hatcheries in the Soviet Far East which produce mainly pink salmon and fall chum salmon. As an experiment the eggs of masu, coho, chinook, and red salmon were incubated in small quantities. There are 18 hatcheries in the Sakhalin region (Figure 1), four in the basin of the Amur River and one on Kamchatka. Information on the quantity of fry released in 1978 is given in Table 1. Ninety-four percent of all hatchery salmon are released in the Sakhalin region, the most developed one in the Soviet Far East, 3.6 percent in the Amur River and 2.4 percent in Kamchatka.

The Sakhalin hatcheries are identified in Table 1. Table 2 gives information on the chum and pink releases for 1948 to 1978 by the basic geographical areas of the Sakhalin region. The area from the Cape of Elizabeth to Terpenya Cape is designated northeastern Sakhalin, from

Table 1. Information of Pacific salmon fry released from the USSR hatcheries in 1978.

Name of the hatchery	Number of the released fry, mln	
	pink salmon	chum salmon
Ado-Tymovsky	—	53.9
Pobedinsky	19.1	0.6
Buyuklovsky	26.9	13.8
Pugachevsky	51.8	—
Sokolovsky	66.7	32.0
Bereznyakovsky	56.1	—
Lesnoy	29.6	6.6
Okhotsky	—	27.0
Anivsky	8.7	11.4
Taranaisky	26.9	9.8
Vatutinsky	1.4	—
Yasnomorsky	—	16.6
Sokolnikovsky	—	22.8
Kalininsky	0.7	87.4
Urozhainy	9.8	0.4
Ainsky	24.9	6.7
Kurilsky	89.0	41.3
Reidovy	56.2	8.7
Teplovsky	—	4.9
Bidzhansky	—	20.3
Gursky	—	3.9
Udinsky	—	2.8
Ushkovsky	—	20.3

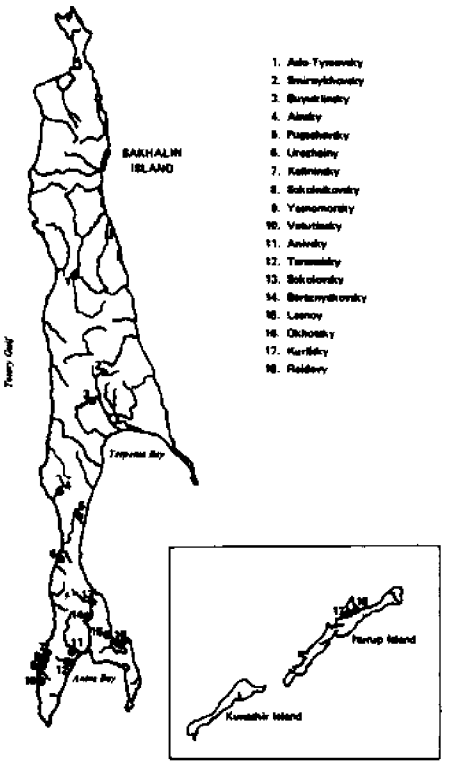

Figure 1. Distribution scheme of the hatcheries in Sakhalin.

Table 2. Information of Pacific salmon fry released by main areas of Sakhalin in 1948-1978.

Area	The released fry of pink, mln	The released fry of chum, mln	Total	Share of area in total release, %		
				pink	chum	Total
Northeastern Sakhalin	9	882	891	0.2	13.4	7.4
Southeastern Sakhalin	2770	2382	5152	50.2	36.3	42.7
Aniva Bay	63	176	239	1.1	2.7	2.0
Southwestern Sakhalin	659	2350	3009	12.0	35.8	24.9
Iturup Island	2012	772	2784	36.5	11.8	23.0
Total	5513	6562	12075	100.0	100.0	100.0

the Terpenya Cape to Aniva Cape as southeastern Sakhalin, from Aniva Cape to Krillion Cape as Aniva Bay, and from Krillion Cape to Lomonon Cape as southwestern. The Iturup area is limited by the territory of this island.

During the period in question, the Sakhalin hatcheries released 12 billion fall chum and pink fry, 46 percent chum and 54 percent pink. The main reproduction area is southeastern Sakhalin. The ratio of the species being reared in this area coincides with the share of chum and pink in the whole region.

In southwestern Sakhalin the share of chum is 78 percent and in Iturup Island 72 percent pink salmon. The share of northeastern Sakhalin salmon reproduction is not large. Ninety-nine percent of the fry released are chum. Chum comprise the main part of the salmon reared in Aniva Bay (74 percent). This area accounts for not more than 2 percent of the total salmon released.

Data have been compared on the quantity of released pink and the quantity of fry migrating downstream to the sea from their natural spawning grounds, southeastern Sakhalin rivers,

Aniva Bay and Iturup Island. The technique for determining downstream migrants of natural origin follows.

Annually pink spawners in southeastern Sakhalin rivers, Aniva Bay and Iturup Island were registered. The share of spawning grounds examined from the air and visually in these areas was in the range of 21 to 58, 22 to 95, and 30 to 98 percent, respectively. The number of fry migrating downstream from natural spawning grounds was calculated by making counts in the rivers under observation and extrapolating the data obtained on the fry from one female for the whole quantity of females. Table 3 shows the number of pink fry in relation to the total number of downstream migrants for the last 15 years. In all areas the tendency toward an increase of the specific weight of the artificial pink has been observed for the last years. The number of hatchery fry is the smallest in the southeastern Sakhalin (17 percent) and the largest on Iturup Island (26 percent).

The question naturally arises as to what is the importance of artificially reared Pacific salmon, and what is the contribution to maintaining the optimum abundance of chum and pink salmon? The results show that the efficiency of rearing chum is rather low (Table 4). The average return for the last 15 years was 0.27 percent in southwestern Sakhalin and in the Naiba River basin (southeastern Sakhalin), 0.28 percent. The maximum figure was 1.76 percent and the minimum 0.01 percent. The assessment of efficiency of chum reproduction in these two areas is rather reliable as in southwestern Sakhalin the entire stock of this species is reproduced artificially and the share of hatchery reproduction in the Naiba River basin approaches 80 percent. The assessment of artificial reproduction of pink salmon was much more difficult as in all hatchery areas pinks naturally occur. Therefore, while assessing the coefficient of return, we've assumed that after downstream migration the mortality for pink salmon of artificial and natural origin is equal. We've calculated the coefficient of pink return in some areas of the Sakhalin-Kuril basin on the basis of the date of fry release from the hatcheries, on the number of fry migrating downstream from natural grounds, and on the results of calculations of returned adult fish of this generation (caught fish plus spawning part of population) (Table 4). The value of return for the last 15 years in all three areas is approximately equal. It fluctuates from 0.7 to 8.7 percent. However, the figures obtained were calculated as previously noted.

It was necessary to obtain more reliable data on pink rearing efficiency. Thus, in 1974 and 1976 at some Sakhalin hatcheries pink fry were marked before release. In 1974 at the Kuril

Table 4. Coefficient of return (percent) of pink and autumn chum in some areas of Sakhalin region for the last 15 years.

Year of return	Pink salmon			Chum salmon	
	Southeast Sakhalin	Aniva Bay	Iturup Island	Southwest Sakhalin	Naiba River
1964	0.5	2.0	2.0	0.65	0.56
1965	2.5	0.3	1.8	0.42	1.76
1966	1.0	1.6	0.7	0.22	0.16
1967	1.6	3.1	1.1	0.23	0.31
1968	1.5	1.1	0.7	0.21	0.55
1969	0.6	2.2	1.7	0.42	0.42
1970	0.3	1.0	1.3	0.20	0.21
1971	1.8	8.7	1.7	0.36	0.22
1972	0.9	0.9	2.9	0.11	0.01
1973	1.9	4.9	2.6	0.17	0.08
1974	2.4	5.7	2.0	0.26	0.03
1975	2.1	4.0	2.1	0.21	0.07
1976	2.8	4.7	2.1	0.13	0.18
1977	7.1	2.9	4.3	0.13	0.07
1978	2.5	2.3	3.5	0.53	0.46
Average	2.1	2.3	2.0	0.27	0.28

Table 3. Share of hatchery pink fry in the total number of downstream migrants of this species in the Southeastern Sakhalin, Aniva Bay and Iturup Island in 1963-1977.

Year	Southeastern Sakhalin			Aniva Bay			Iturup Island		
	Fish released from hatchery (mln)	Downstream migrants from natural spawning grounds	Share of hatchery pink fry (%)	Fish released from hatchery (mln)	Downstream migrants from natural spawning grounds (mln)	Share of hatchery pink fry (%)	Fish released from hatchery (mln)	Downstream migrants from natural spawning grounds (%)	Share of hatchery pink fry (%)
	1	2	3	1	2	3	1	2	3
1963	16	850	2	17	111	13	19	95	20
1964	81	774	9	37	342	10	134	316	42
1965	38	527	7	32	125	20	23	435	5
1966	88	960	8	18	52	26	143	350	41
1967	78	368	17	36	180	17	76	365	21
1968	188	2164	8	48	127	27	134	548	24
1969	108	315	26	17	245	7	88	458	19
1970	191	1113	15	41	72	36	143	590	24
1971	87	325	21	17	37	31	94	360	26
1972	173	1185	13	36	101	26	137	456	30
1973	137	555	20	23	81	22	97	521	19

Hatchery one million fry were marked by cutting the adipose fin. In 1975 at the Iturup fish processing plants and hatcheries 27,000 marked fry (2.7 percent) were recovered. The calculated index of this year's return was 2.1 percent.

In addition it should be noted that 7,000 marked fish were also found in eastern Sakhalin and Aniva Bay. In 1977 the return of marked fish to Iturup Island was 4.7 percent, the calculated index of return was 4.3 percent. Thus, double testing of the calculated return index by registration of tagged fish has shown comparable results. Therefore, we believe that the figures of return in Table 4 reflect the actual situation.

A comparison of the efficiency of chum and pink reproduction indicates a preference of pink rearing. Thus, lately the main stress is on the rearing of this species in the Sakhalin region. The results of 1976 tagging showed fish returns to Ainsky, Sokolovsky, Lesnoy and Kuril hatcheries were quite different. From an inspection of recovered pink salmon, 0.11 percent were marked at Ainsky, 0.09 percent at Sokolovsky, 10.4 percent at Lesnoy and 4.7 percent at the Kuril Hatchery. The average coefficient of pink return was 3.4 percent. These results are assumed to be preliminary, requiring additional tests. Hatchery identification of fry was determined by clipping fins in the following manner: at the Ainsky Hatchery, adipose and right ventral fins; at Sokolovsky, adipose and left ventral fins; at Lesnoy, the adipose fin; and at Kuril, the adipose and dorsal fins. Low pink return to the first two hatcheries and a high return rate to Lesnoy Hatchery can be explained by the capacity of the ventral fin to regenerate. If further tests show that our assumption is wrong, it would be necessary to study the reasons of low efficiency of the experiments and use so far as average value of return obtained at these four experiments. This value shows the efficiency of artificial pink rearing is rather high.

Taking into account the promising salmon fisheries, a draft plan of fisheries development in the region with 3 billion fry annually to the year 2000 has been submitted for discussion to scientists, fishermen and fishery departments. It is planned to increase the release of pink salmon to 1.9 billion, chum to 900 million, masu to 65 million, coho to 55 million, and red to 50 million fry. For other far eastern regions it is planned to increase the salmon fry release by 1 billion. It is anticipated that during discussions, these figures would be changed according to species composition. Nevertheless, a program leading to a considerable increase of salmon fisheries in the Soviet Far East has been adopted and will be realized during the next 20 years.

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Plastic Matrix Substrates for Incubating Salmon

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Abstract

Fry resulting from incubation with smooth-bottomed incubators containing no matrix substrate often are smaller, suffer greater mortality, exhibit anomalous yolk sac absorption, or have a combination of these problems. In contrast, Atlantic salmon (*Salmo salar*) incubated in units containing Intalox Saddles were 45 percent heavier, had normal yolk sac absorption, high survival, and fed well after emergence.

Astroturf, Intalox Saddles, and gravel were compared as substrates for five species of Pacific salmon. Emergent fry from saddles and gravel were generally larger, survived better in incubators and emerged at times more closely resembling those of creek fry. Fry incubated in saddles had a significantly shorter period of emergence than those from other substrates. Saddle-incubated fry survived to adult at about the same rate as gravel-incubated fry. Moderate to deep matrix incubators with saddles have much greater egg and alevin capacities per surface area of floor space than incubation systems using gravel in boxes or raceways.

Plastic Matrix Substrates for Incubating Salmon

My initial interest in incubation research for the improvement of salmonid culture technology was stimulated in 1971 when I found that Atlantic salmon (*Salmo salar*) hatcheries typically lost 20 to 50 percent of their alevins or swimup fry. Those fry that did not die immediately were often up to 40 percent smaller than their natural river-produced counterparts. Marr (1963 and 1965) reported that light and surface contour in incubators influenced the behavior, development, and growth of trout and salmon embryos. Personal experience made it clear to me that traditional hatchery incubation methods produce salmonid fry of less than optimal quality and survival. Trough baskets, trough trays, stacked tray cabinets, and hatching jars all may produce fry of inferior quality. Undesirable characteristics may include smaller size at fry emergence, abnormal yolk sac development, poor feeding response and crowding behavior (Leon, 1975 and 1979).

As Marr pointed out, providing additional surfaces, other than the flat bottoms of incubators, can improve fry quality. In nature, gravel provides those surfaces. Progressive culturists adapted this natural substrate to incubation boxes, as widely used in Canada, and to raceway alevin development that is common in Japan and the Soviet Union. Others have used more exotic substrates such as Astroturf. I felt that Intalox Saddles, a matrix substrate normally used to mix liquids and gases, would not only provide the required surface contours but would also increase potential loading density of eggs and alevins, cause better water distribution in incubators, and give the culturist a lighter material to work with.

Results

Saddles were first tested at several depths and loading densities with Atlantic salmon. When saddles were used, yolk sac development was normal, survival and growth were

significantly increased, and feeding behavior was much superior to fry produced in incubators without saddles (Leon, 1975 and 1979).

In 1976, 1977 and 1978 saddles were compared to gravel, Astroturf, flat screens, and corrugated screens for use in incubators for chum salmon (*Oncorhynchus keta*), pink salmon (*O. gorbuscha*), sockeye salmon (*O. nerka*) and coho salmon (*O. kisutch*). Saddles generally produced the highest quality fry (Tables 1, 2, and 3), in addition to producing a fry emergence timing very close to that of river-incubated fry. Another benefit was that saddles caused a much shorter emergence period (Figure 1). Chum and pink salmon had the most positive response to the saddle substrate. Coho and sockeye salmon also did better with this substrate but the benefits were less striking.

Table 1. Loading densities, emergence weight, percentage weight difference, and eyed egg to fry survival of coho and pink salmon incubated at the Starrigavan Hatchery.

Substrate	Species	Eggs/cc (in³)	Weight (mg)	%△[1]	% egg-fry
Turf	Coho	.43 (7)	363a[1]		90+
Saddles	Coho	.43 (7)	391a[1]	8	90+
Heath[2]	Coho	.43 (7)	412b[1]		
Heath[3]	Coho	.43 (7)	398b[1]	3.5	90+
Saddles	Pink	1.35 (22)	289		92

[1]Percentage difference refers to those weights followed by the same letter.
[2]These Heath Trays contained either saddles or corrugated screens.
[3]These Heath Trays contained no substrate other than the factory-supplied screens.

Table 2. Comparison of emergence weight and survival of pink salmon incubated at the Auke Bay Laboratory Hatchery with three substrates.

Substrate	Weight (mg)	% egg-fry
Turf	270	86.6
Gravel	271	88.9
Saddles	288	96.6

Table 3. Comparison of emergence weight and eyed egg to fry survival of chum salmon incubated at the Beaver Falls Hatchery with three substrates and two loading densities.

Substrate	Eggs/cc (in³)	Weight (mg)	% egg fry
Turf	.43 (7)	399	38.5
	.55 (9)	432[1]	24.2
Gravel	.43 (7)	432	69.1
Saddles	.43 (7)	451	81.2
	.55 (9)	447	74.5

[1]High mortality in this lot may have affected emergence weight, i.e. fewer live alevins competing for the same space.

The increase in efficient use of hatchery floor space is astonishing when the use of tiered, deep matrix incubators with saddles is compared with other fish incubation systems in the world. Between 500,000 and 600,000 fully-developed chum salmon fry per square meter of floor space are presently produced by this method as compared to less than 100,000 fry per square meter with most other methods. Of course, sockeye and pink salmon fry can be produced at proportionately higher numbers per surface area because their eggs are smaller and have lower metabolic requirements.

Evidence from the first few years of adult salmon recoveries indicates that fry from saddle-incubated salmon survive to adulthood at about the same rate as gravel-incubated fry. Examples of adult salmon production from fry incubated in saddles are shown in Table 4.

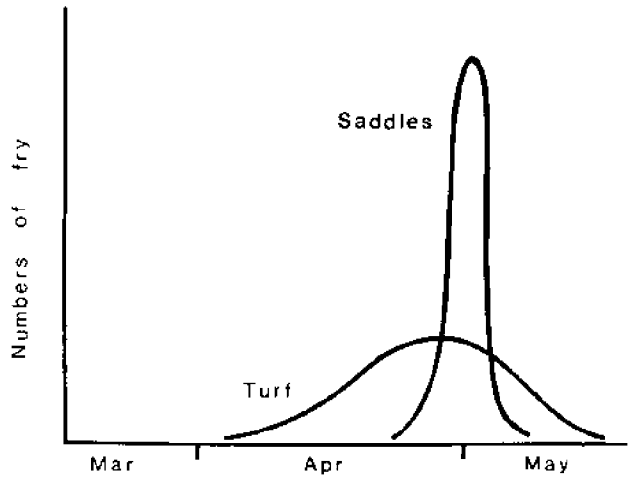

Figure 1. Comparison of emergence timing of salmon fry incubated in either Intalox Saddles or Astroturf.

Table 4. Pink salmon fry to adult survival at two facilities using saddles at various loading densities.

Hatchery	Eggs/cc (in^3)	Egg/cm^2 (in^2)	% survival
Kitoi	.76 (12.4)	26.7 (172)	1.2 - 1.5
Tutka	1-2 (16-32)	29.9 (192)	4.7 - 9.9

Conclusion

During the next few years, more definitive information on survival to adulthood will be obtained from the expanded use of saddles. At this time, the conclusion is that our saddle incubation methods produce a greater number of healthy fry per hatchery surface area than any other known technique.

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Pacific Salmon Alevin Incubation Densities and Alevins/dm² Incubator Area in Intalox Saddle Plastic Substrate at Alaskan Hatcheries

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Abstract

Northeastern Pacific salmon alevins are incubated in Intalox Saddle plastic substrate (2.5 cm size) at Alaska Department of Fish and Game salmon hatcheries.

Salmon alevin densities tested have ranged from 121 to 2,884 alevins/dm³ of incubator volume with saddles. The number of alevins tested/dm² incubator area with substrate has ranged from 156 to 6,817. Substrate depths have ranged from 5 to 90 cm.

Eyed egg to fry survival; salmon fry quality in terms of weight, length, and development index (K_D); and survival to adulthood are summarized for Intalox Saddle substrate and some comparisons are made between saddle, hatchery gravel, and river-incubated fry.

Introduction

Gravel substrate in hatchery incubators is a proven producer of adult salmon (Bailey, et. al.,1976; Bams, 1972 and 1974). Intalox Saddle substrate is also a proven producer of adult salmon.

This report presents eyed egg to fry survival; salmon fry quality in terms of weight, length, and/or developmental index (Bams, 1970); and survival to adulthood relative to alevin densities and alevins/dm² incubator area in Intalox Saddle substrate. Various alevin densities and numbers/dm² incubator area in saddles are compared with hatchery gravel and river gravel[1] relative to fry quality and survival to adulthood.

Eyed Egg to Fry Survival

Eyed egg to emergent salmon fry survivals in Intalox Saddle substrate were examined relative to incubator volume (dm³) and area (dm²). Survival data were obtained for all five species of eastern Pacific salmon.

Survival is not apparently a function of the number of alevins[2] (eyed eggs)/dm³ of substrate[3]

[1]River gravel refers to gravel substrate incubation in streams but not in hatcheries, spawning channels, or incubation channels.

[2]In this report, "alevins" and "eyed eggs" are used interchangeably. Eyed eggs, but not alevins, were counted during the incubation period. The number of just-hatched alevins is essentially equal to the number of eyed eggs seeded.

[3]dm³ of substrate refers to the incubator volume (dm³) which was loaded with substrate.

within the range of alevin densities (121 to 2,884/dm³) examined (Figure 1). The noteworthy consideration is that ≥ 90 percent survival is possible with densities ranging from 120 to 2,825 alevins/dm³. Furthermore, ≥ 95 percent survival is documented for densities ≤ 2,000.

Survival is also not apparently a function of the number of eyed eggs loaded per dm² of incubator area with substrate within the range of eyed eggs per dm² (156 to 6,817) examined (Figure 2). High survival (≥ 90 percent) is possible at all loads/dm² observed and ≥ 95 percent survival is documented at loadings/dm² ≤ 4,100.

Since high survival can be attained over a wide range of alevin densities and loadings/dm², high survival should not be a function of substrate depth, as long as there is enough substrate to cover the alevins.

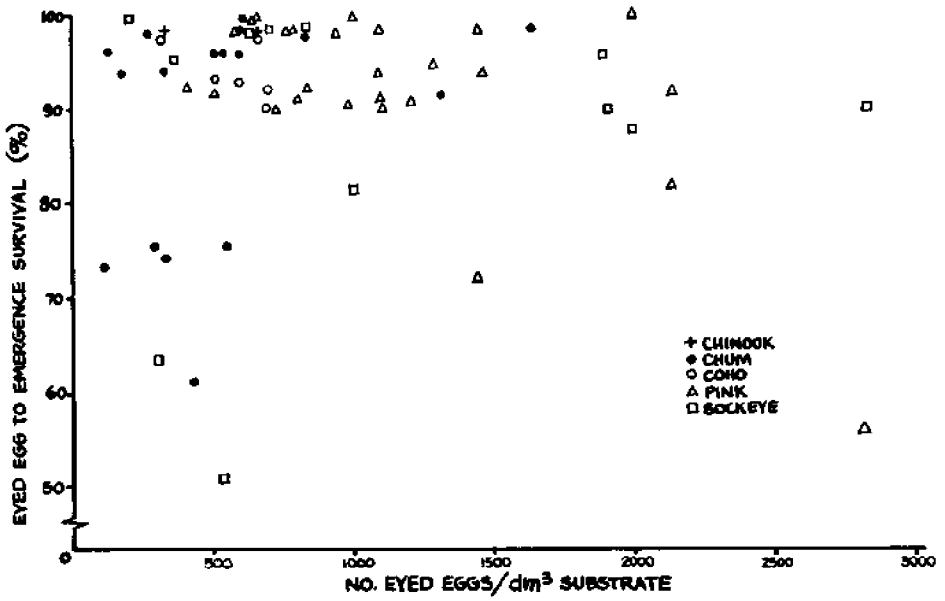

Figure 1. Pacific salmon eyed egg to emergent fry survivals relative to densities of eggs incubated in Intalox Saddle substrate. (Most data are from Alaska Department of Fish and Game hatcheries. Some pink salmon data were provided by Mr. Jack Bailey, National Marine Fisheries Service, Auke Bay, Alaska 99821.)

Alevin Density—Areal Load Relationship

The number or weight of eyed eggs, and subsequent alevins, seeded in a unit volume of substrate, has generally not represented the true density for two reasons:

1) Observations within incubators show that the great majority of alevins migrate to the bottom of the substrate, regardless of substrate depth.
2) Most incubators have utilized much greater depths of substrate than necessary to cover the alevins.

Therefore, true density must be distinguished from apparent density. In this report, apparent rather than true density was used in discussing alevin to emergent fry survival. True density is difficult to determine since: 1) not all (100 percent) alevins migrate to the bottom of the substrate; and 2) true density changes close to the time of emergence.

In my opinion, areal load, i.e., the number of eyed eggs loaded per unit incubator (dm² in this report) with substrate is a more important concept than apparent or true density, provided that substrate is deep enough to cover the alevins. Areal load does not change from the time eyed eggs are seeded to the time of fry emergence.

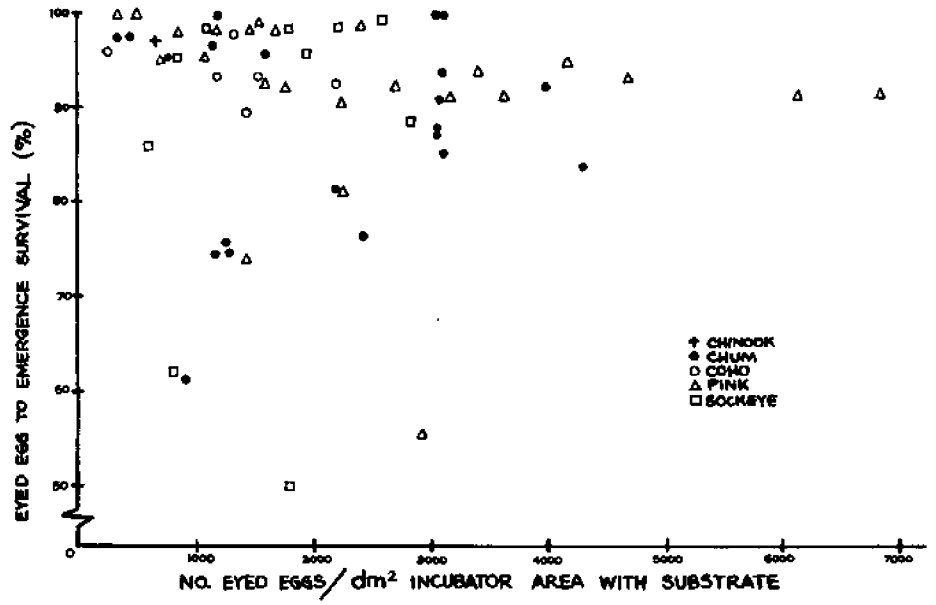

Figure 2. Pacific salmon eyed egg to emergent fry survivals relative to incubator area (dm²) for eyed eggs incubated in Intalox Saddle substrate. (Most data are from Alaska Department of Fish and Game hatcheries. Some pink salmon data were provided by Mr. Jack Bailey, National Marine Fisheries Service, Auke Bay Laboratory, Auke Bay, Alaska 99821.)

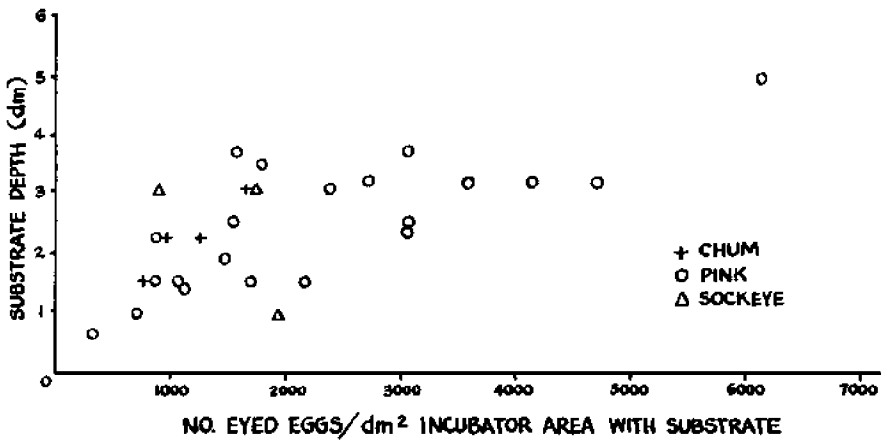

Figure 3. Intalox Saddle substrate depth relative to number of Pacific salmon eyed eggs per incubator area (dm²) when Intalox Saddle produced fry weight and/or length equalled or exceeded that of hatchery gravel produced fry or river fry. (Most data are from Alaska Department of Fish and Game hatcheries. Some pink salmon data are from Mr. Jack Bailey, National Marine Fisheries Service, Auke Bay Laboratory, Auke Bay, Alaska 99821.)

Substrate depth was examined relative to areal load (number eyed eggs/dm²) in those cases when saddle fry weight and/or length equalled or exceeded that of hatchery gravel or river fry (Figure 3). Saddle substrate depth is not a major factor in determining fry quality. A substrate depth of ≤ 3.2 dm produces high quality fry over quite a range of areal loads (310 to 4,664 alevins/dm²). As little as 1 dm of substrate depth will support a high loading of 1,929 alevins/dm².

Fry Quality Relative to Areal Load in Intalox Saddle Substrate

Chum, pink, and sockeye salmon fry at various areal loads in saddles were compared with hatchery gravel and river fry, relative to fry quality.

Chum fry from areal loads as high as 1,631 alevins/dm² in saddles were as large or larger than hatchery gravel or river fry (Figures 4 and 5). In Figure 5, saddle fry apparently have more yolk reserves ($> K_D$) than river fry.

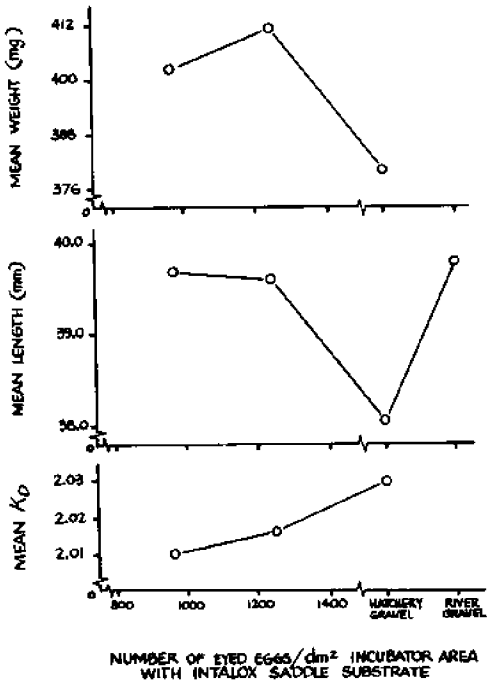

Figure 4. Chum salmon emergent fry weight, length, and development index (K_D) relative to number of eyed eggs per incubator area with Intalox Saddle substrate and relative to hatchery gravel and river gravel. (Data are from Beaver Falls Hatchery, 1976 Brood Year.)

Pink fry from areal loads up to 6,117 alevins/dm² in saddles were larger than hatchery gravel or river fry (Figures 6, 7, 8, and 9). Saddle fry have slightly more yolk reserves than hatchery or river fry.

At two hatcheries where a wide range of areal loads occurred, pink fry weight tended to decrease as areal loads increased (Figures 7 and 9). Saddle fry weights were still generally larger than those of gravel fry.

The small amount of data on sockeye fry indicate that fry from areal loadings up to 1,929 alevins/dm² can be larger than gravel fry (Figure 10).

Fry Biomass Relative to Areal Load in Intalox Saddle Substrate

Alevin biomass is more important than numbers for certain aspects of incubation, such as dissolved oxygen consumption and ammonia excretion determinations. Therefore, this report includes data relating emergent fry mean weight to emergent fry biomass/dm² of incubator area with substrate (Figure 11).

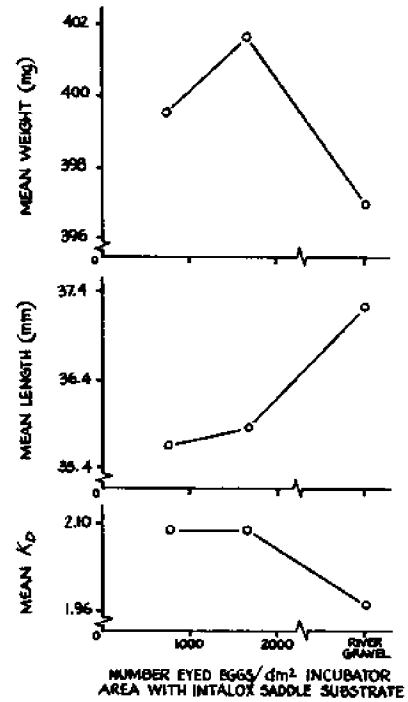

Figure 5. Chum salmon emergent fry weight, length, and development index (K_D) relative to number of eyed eggs per incubator area with Intalox Saddle substrate and relative to river gravel. (Data are from Russell Creek Hatchery, 1979 brood year.)

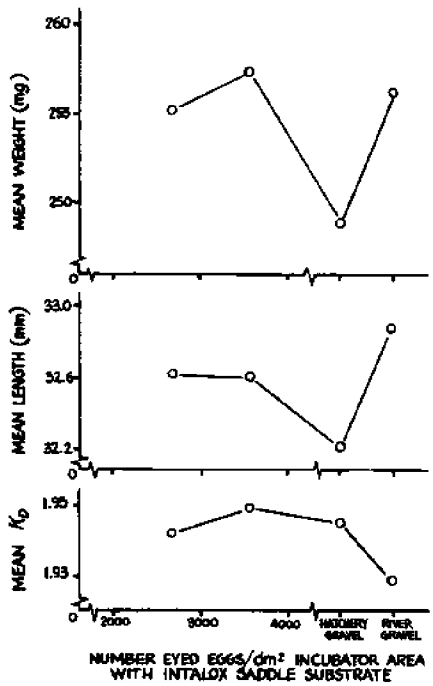

Figure 6. Pink salmon emergent fry weight, length, and development index (K_D) relative to number of eyed eggs per incubator area with Intalox Saddle substrate and relative to hatchery gravel and river gravel. (Data are from Kitoi Bay Hatchery, 1977 brood year.)

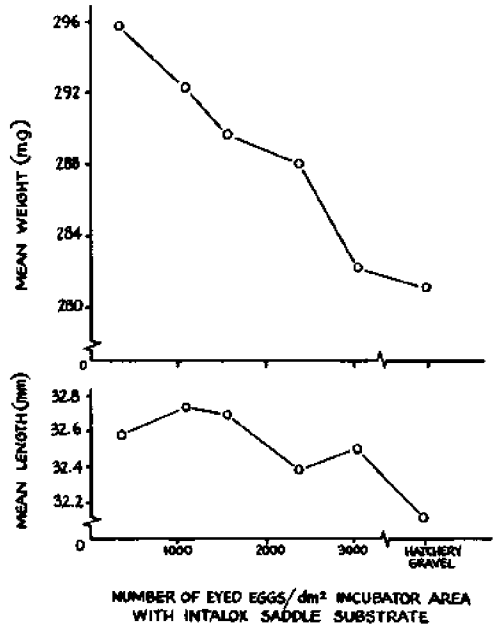

Figure 7. Pink salmon emergent fry weight and length relative to number of eyed eggs per incubator area with Intalox Saddle substrate and relative to hatchery gravel. (Data are from Auke Bay Hatchery, 1976 brood year and were provided by Mr. Jack Bailey, National Marine Fisheries Service, Auke Bay Laboratory, Auke Bay, Alaska 99821.)

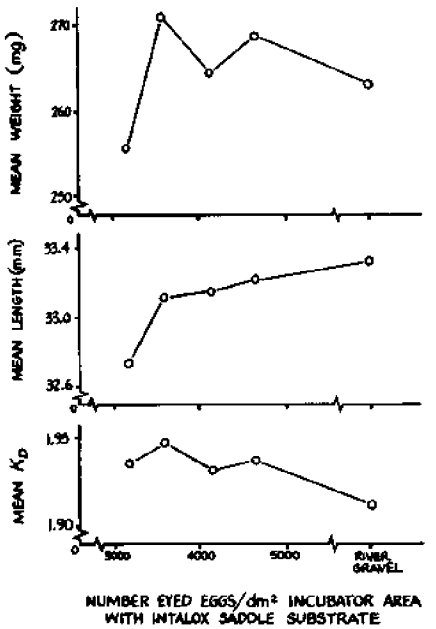

Figure 8. Pink salmon emergent fry weight, length, and development index (K_D) relative to number of eyed eggs per incubator area with Intalox Saddle substrate and relative to river gravel. (Data are from Kitoi Bay Hatchery, 1978 brood year.)

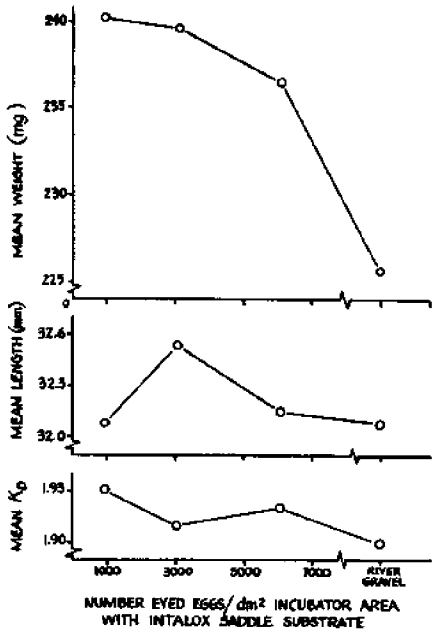

Figure 9. Pink salmon emergent fry weight, length, and development index (K_D) relative to number of eyed eggs per incubator area with Intalox Saddle substrate and relative to river gravel. (Data are from Tutka Bay Hatchery, 1978 brood year.)

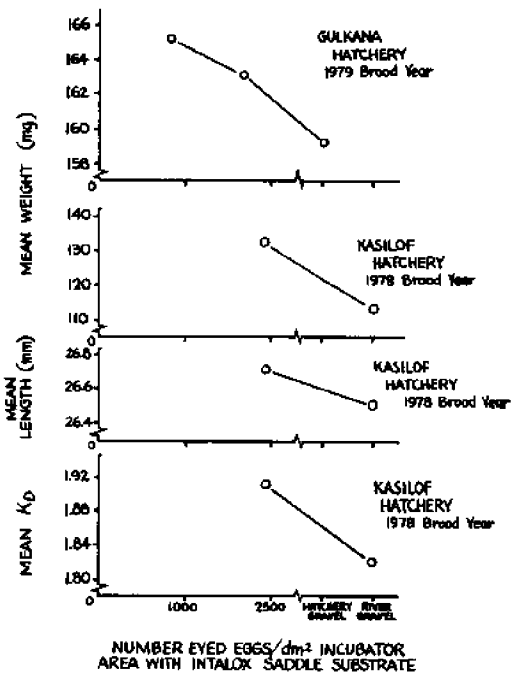

Figure 10. Sockeye salmon emergent fry weight, length, and development index (K_D) relative to number of eyed eggs per incubator area with Intalox Saddle substrate and relative to hatchery and river gravel.

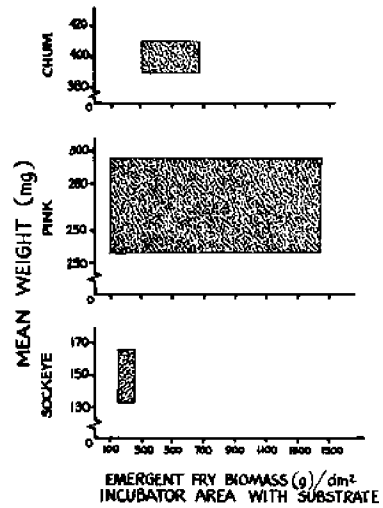

Figure 11. Pacific salmon fry biomass per incubator area with Intalox Saddle substrate that should produce fry equal to or larger than hatchery gravel or river fry in weight and/or length. (Most data are from Alaska Department of Fish and Game hatcheries. Some pink salmon data were provided by Mr. Jack Bailey, National Marine Fisheries Service, Auke Bay Laboratory, Auke Bay, Alaska 99821.)

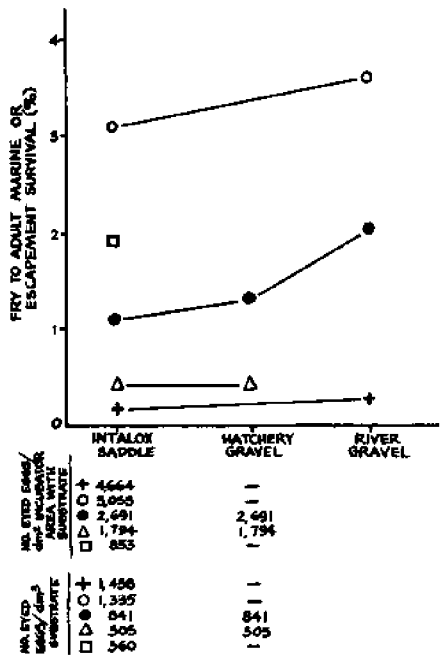

Figure 12. Pink salmon unfed fry to adult marine or escapement survival relative to number of eyed eggs per incubator area and eyed egg densities in substrate. (Data are from Alaska Department of Fish and Game hatcheries.)

Fry to Adult Survival

Pink salmon fry to adult survivals for saddle, hatchery gravel, and river fry are depicted in Figure 12. In this same figure, apparent eyed egg densities (number/dm^3) and areal loads (number/dm^2) are presented for saddle and hatchery gravel fry.

Saddle fry to adult survival is good relative to that for hatchery gravel and river fry. Furthermore, the greater egg to fry survival for saddle fry than for river fry will result in a greater adult return per spawner for saddle fry.

Conclusion

High eyed egg to fry survival, high quality fry, and good fry to adult survival are realized at high alevin densities and areal loads in Intalox Saddle substrate.

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Effectiveness of Pink Salmon Reproduction at the Hatcheries of the Sakhalin Region

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In 1979, investigations were carried out to determine the effectiveness of the Sakhalin hatcheries by marking the released salmon fry and registering the returned adults. The long-term program of the present investigations is to provide fish marking in all existing hatcheries where artificial culturing combines with natural reproduction. Experience has shown the fin clip method to be a reliable way of marking fry and has made it possible to define the coefficient of fish return to four hatcheries (Roukhlov and Ljubaeva, 1977; Roukhlov and Ljubaeva, 1980).

The results of pink salmon registration, marked in 1978, were used for this paper. Marking was done as follows: Adipose and dorsal fins of 300,000 fry were clipped in the Taranaisky Hatchery; adipose fins of 500,000 fry were clipped in the Sokolovsky Hatchery, and adipose and right ventral fins of 306,000 fry were clipped in the Kurilsky Hatchery.

Registration of returned marked fish was made during the whole 1979 fishing season at the largest shore bases of Sakhalin and Kuril Islands. Thirty-four percent of the fish from the total catch were examined. Daily returns were calculated for each of the separate marked groups. The total figures for the whole fishing season were summarized by days for a given fishing area. There were six fishing areas; their borders and names are given in Figure 1.

Pink salmon entering the rivers were observed at spawner collecting fences. Forty-one percent of the entering spawners were examined for markings in Bystraya and Taranay (Aniva Bay), Lesnaya and Bolshoy Takoy (southeast Sakhalin), and Reidovaya and Kurilka (Iturup Island) rivers.

For the total catch in the Sakhalin-Kuril basin, the number of fish with adipose fin clips was 15,294; adipose and dorsal fin clips, 1,866; and adipose and right ventral fin clips, 17,363. The distribution of marked fish in areas of registration is given in absolute figures in Table 1. The coefficient of pink salmon return released from the Taranaisky Hatchery was 0.6; from Sokolovsky, 3.1; and from Kurilsky, 5.7. The average coefficient of fish return for these three hatcheries was 4.0 percent. The bulk of marked fish was found in catches from fixed nets. The number of discovered marked fish for Sokolovsky Hatchery, Taranaisky Hatchery, and Kurilsky Hatchery was 87 percent, 95 percent and 90 percent, respectively (Table 1).

The Kurilsky and Taranaisky hatchery marked fish were caught mainly in the regions nearest their release. More than 90 percent of the pink salmon from Taranaisky Hatchery were caught in Aniva Bay and 99.9 percent of individuals marked in Kurilsky Hatchery returned at maturity to the coast of Iturup Island.

Near southwestern Sakhalin one could find pink salmon from all regions where markings

Table 1. Distributioin of tagged pink by areas of calculation works in 1979.

Area of calculation works	Share (%) of observed fish in total catch	Share of recovered tagged species (%) in total number of fish			Coefficient of return of tagged fish		
		Sokolovsky	Taranaisky	Kurilsky	Sokolovsky	Taranaisky	Kurilsky
Southeastern Sakhalin	40.7	2.26	0.48	0.01	0.069	0.003	0.001
Aniva Bay	37.8	1.54	90.19	—	0.047	0.561	—
Okhotskoye-Lesnoye area	39.3	9.05	1.71	0.02	0.277	0.011	0.001
Starodubskoye area	46.4	11.95	2.20	0.02	0.366	0.014	0.001
Terpenya Bay	46.9	1.67	0.11	—	0.051	0.001	—
Total on Sakhalin	44.0	26.47	94.69	0.05	0.810	0.590	0.003
Iturup Island (Prostor, Kurilsky, Kuybyshevsky bays)	26.0	60.60	0.16	89.16	1.854	0.001	5.059
Total in sea	33.9	37.07	94.85	89.21	2.664	0.591	5.062
Bolshoy Takoy River	44.1	10.57	—	0.01	0.323	—	0.001
Ochepukha River (Lesnaya)	29.6	1.19	1.10	0.02	0.035	0.007	0.001
Bryanka River (Bystraya)	85.7	0.17	1.70	—	0.005	0.011	—
Taranya River	92.3	—	2.35	—	—	0.015	—
Reidovaya River	51.9	0.31	—	—	0.010	—	—
Kurilka River	30.4	0.69	—	10.67	0.021	—	0.611
Total in rivers	41.4	12.93	5.15	10.79	0.395	0.033	0.613
TOTAL	34.4	100.00	100.00	100.00	3.059	0.624	5.675

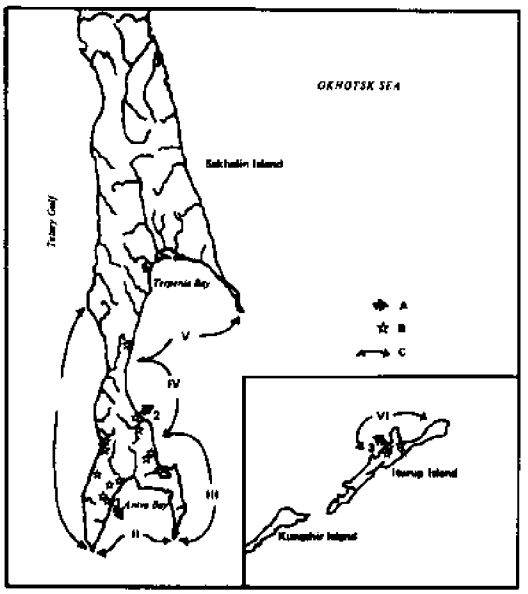

Figure 1. The areas of release and calculation of the tagged humpback.

A — areas of release
1 — Taranaisky Hatchery
2 — Sokolovsky Hatchery
3 — Kurilsky Hatchery
B — areas of calculation of the tagged fish
C — borders of the areas
I — Southwestern Sakhalin
II — Aniva Bay
III — Lesnoy-Okhotskoye area
IV — Starodubskoye area
V — Terpenya Bay
VI — Iturup Island

were made. Pink salmon from the Kuril Hatchery were not found in Aniva Bay. Only one individual that had been marked at Taranaisky Hatchery was found in Iturup Island.

The majority of pink salmon marked at the Sokolovsky Hatchery was found in the coastal waters of Iturup Island. Moreover, Sokolovsky's fish were caught earlier than fish with Kurilsky Hatchery marks. The highest coefficient of marked fish return was observed at the Kurilsky Hatchery. Analogous investigations were carried out here in 1974 and 1977 and in all cases the effectiveness of this hatchery was high. In 1974, fish return was 3.4 percent and in 1977, 4.7 percent. The results of marking are proved by significant catches and by the number of pink salmon spawners run to spawning grounds. Nevertheless, the distribution of marked fish in fisheries areas in various years was different. In 1974, 0.7 percent of individuals from the total number of marked fish from the Kurilsky Hatchery were caught near the Sakhalin coast. In 1977, the figure was 0.005 percent and in 1979, the figure was 0.003 percent. Redistribution was also observed in pink salmon marked in the Sakhalin Hatchery. Thus, in 1977, 40 percent of fish released from the Lesnoy Hatchery was found in fixed nets off Iturup Island. But usually pink salmon from the nearest Sokolovsky Hatchery were caught close to Sakhalin Island. Near Iturup Island 4 percent of the pink salmon with marks of this hatchery were found (Table 1). In 1979, this ratio changed. Sixty-one percent of pink salmon with Sokolovsky Hatchery marks were found off Iturup Island and only 27 percent were found in Sakhalin fixed net fishing. Significant differences were observed in distribution of fish released from Sokolovsky Hatchery in Aniva Bay. In 1979, only 1.5 percent of individuals with marks of this hatchery were found there and in 1977, it was 28.7 percent. In the "Starodubskoe" district their number decreased more than four times, and in the "Okhotskoe-Lesnoe" district it was three times more.

Distribution of fish entering the rivers for spawning also changed. The coefficient of fish returning to the Kurilka River which were released from the Kurilsky Hatchery in 1979 was higher than in 1977, that is 0.61 and 0.16 percent, respectively. In 1977, pink salmon with Sokolovsky marks were not found in the rivers of Iturup Island. In 1979, 105 pink salmon entered the Kurilka River and 48 entered the Reydovaya River. But in 1977, 797 individuals with Lesnoy Hatchery marks were registered in the Kurilka River.

Comparing the results of the investigations in 1977 and 1979 one can expose not only differences but common characteristics of fish distribution in investigated areas (Table 2). Pink salmon reproducing in the southeast Sakhalin hatcheries were caught in significant numbers in coastal waters of Iturup Island. The catch of pink salmon released from Kurilsky Hatchery close to Sakhalin is insignificant and during three years of observations, it did not exceed 0.7 percent of the total number of marked fish. The majority of individuals with cut fins was caught in rivers with hatcheries and in fishing areas adjacent to these rivers. Pink salmon marked in 1979 at the Sokolovsky Hatchery were an exception.

During 1977 and 1979 fishing seasons, the overwhelming majority of marked individuals was found in catches from fixed nets. Pink salmon released from the southeast Sakhalin hatcheries returned for spawning to the Iturup Island coast somewhat earlier as compared to those from hatcheries of this island. In 1979, their arrival was observed between 20 and 25 June. The local fish appeared from 20 to 25 August. Both in 1977 and in 1979, the peak spawning run of marked pink salmon from the southeast Sakhalin hatcheries to the Iturup Island coast was earlier (for almost a decade) as compared to Kuril pink salmon. Probably this is caused by the fact that the Sakhalin pink salmon, in order to reach the spawning grounds, run earlier. However, provided Sakhalin pink salmon entrance to the rivers of Iturup Island is proved, the question of what part of this fish is anadromous is still under consideration. At present we can only ascertain that migrating Sakhalin pink salmon along the Iturup Island coast are caught in that fishery during some years.

Table 2. Distribution of tagged pink (%) by a number of recovered fish by areas in 1977 and 1979.

	Sokolovsky Hatchery		Lesnoy Hatchery	Kurilsky Hatchery	
Areas of calculated works	1977	1979	1977	1977	1979
Southwestern Sakhalin	2.38	2.26	1.06	0.05	0.01
Aniva Bay	28.71	1.54	6.02	0.01	—
Okhotskoye-Lesnoye area	3.15	9.05	33.95	0.01	0.02
Starodubskoye area	51.57	11.95	12.20	0.02	0.02
Terpenya Bay	1.19	1.67	0.09	0.01	—
Total on Sakhalin	90.14[1]	26.47	54.94[2]	0.10	0.05
Iturup Island	3.78	60.60	40.01	96.55	89.16
Total in sea nets	93.93	87.07	94.95	96.65	89.21
Bolshoy Takoy River	4.15	10.57	0.22	0.002	0.01
Ochepukha River (Lesnaya)	1.52	1.19	4.07	0.01	0.02
Bystraya River (Bryanka)	—	0.17	—	—	—
Taranay River	—	—	—	—	—
Reidovaya River	—	0.31	—	—	—
Kurilka River	—	0.69	0.76	3.34	10.76
Total in rivers	6.07	12.93	5.05	3.35	10.79

[1]Including the area of "Eastern Sakhalin" — 3.14.
[2]Including the area of "Eastern Sakhalin" — 1.62.

Section III
Enrichment of Lakes for Aquaculture

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Aug. 1980, Anchorage, Alaska

Effects of Fertilization of Little Togiak Lake on the Food Supply and Growth of Sockeye Salmon

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Abstract

Juvenile sockeye salmon (*Oncorhynchus nerka*) in the Wood River lake system exhibit density-dependent growth and are among the smallest smolts produced in Bristol Bay. We hypothesized that an increase in growth would be followed by an increase in survival and hence an increase in the abundance of returning adults. Little Togiak Lake (6 km²) was annually treated with various amounts of diammonium phosphate (220 to 1,780 kg) during the summers of 1974 to 1978 and the effects on phytoplankton, zooplankton, chironomids, and fish growth were monitored. In each year that phosphates were applied, the biomass of phytoplankton increased within a few days and the increase was approximately proportional to the density applied. Significant increases in abundance of zooplankton and chironomids during June to September were observed in some, but not all years; however, following each year of fertilization, the smolts that migrated from Little Togiak Lake were larger than those from the other lakes in the system, indicating that growth was enhanced by fertilization.

Introduction

The commercial fishery for sockeye salmon (*Oncorhynchus nerka*) in the Nushagak District of Bristol Bay developed in the late 1880s. Following the build-up of the fishery, there was a 20-year period when the annual catches averaged 5 million fish and then a 30-year period when the catches averaged 3 million fish. Since 1949, the catches have averaged about 1 million. Comparable declines in catches did not occur in the other fishing districts in Bristol Bay and the decline in the Nushagak catches appears to have been caused by a decline in the productivity (return per spawner) of the Wood River stock, which in the most recent period has produced the majority of fish in the Nushagak District. The level of escapements to the Wood River lake system during the three periods of the fishery did not change significantly, although there has been considerable annual variation (Mathisen, 1971).

The Fisheries Research Institute began investigations of the sockeye salmon in the Wood River lakes in 1946. By the end of the 1960s, it was evident that: 1) smolts migrating from Wood River in recent years were the smallest in Bristol Bay and were smaller than smolts that migrated from Wood River in the early 1900s; 2) growth of juvenile sockeye was density-dependent; 3) the standing stock of zooplankton (their principal food) was inversely related to the abundance of juveniles; 4) primary productivity was low and did not vary greatly from year to year; and 5) phosphorus was the most likely nutrient that limited primary production (Burgner, 1964; Burgner, et al., 1969; Gadau, 1966; and Rogers, 1968, 1973 and 1977). Therefore, in 1970 we fertilized a small bay (0.6 km²) in Lake Aleknagik (Figure 1) with a commercial diammonium phosphate fertilizer to determine if primary and secondary productivity could be increased. The standing crop of phytoplankton increased in 1970 and the abundance of zooplankton and chironomids increased in 1971 (Rogers, et al., 1973).

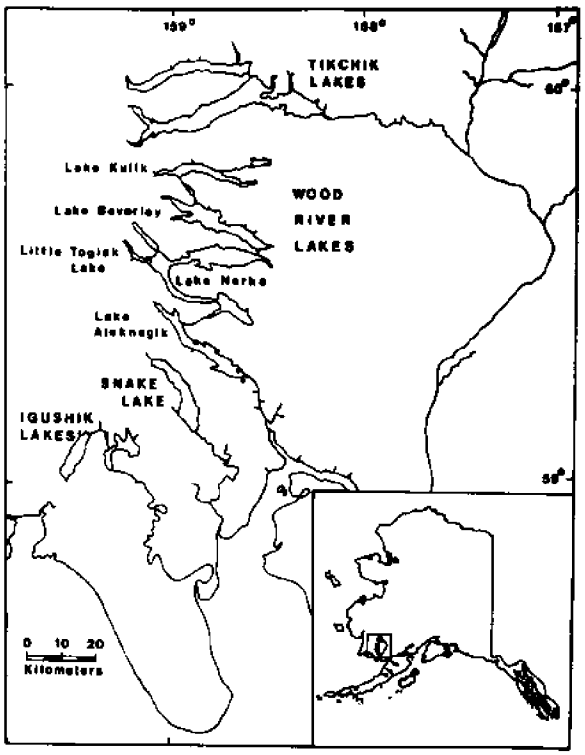

Figure 1. Lakes of the Nushagak District of Bristol Bay.

Little Togiak Lake was chosen for experimental fertilization because it is small (6 km²), has a history of high fish density and poor growth of juvenile sockeye salmon, and the abundance and size of smolts and the abundance of returning adults could be measured easily. Intensive limnological measurements were begun in 1973 and variable amounts of fertilizer were added to the lake during the summers of 1974 to 1978. A final evaluation of the effects on the production of sockeye salmon will not be made until 1982. This report is a summary of the results to date. Detailed methods and results are given in B. Rogers (1979), D. Rogers (1979) and Hardy (1979).

Methods

Since the experimental fertilization of Little Togiak Lake was conducted under natural, and thus uncontrolled environmental conditions, it was important that we had several long-term measurements of environmental parameters so the effects of fertilization could be separated from natural annual variation. Although intensive sampling in Little Togiak Lake did not begin until 1973 for some parameters, such as chlorophyll *a* and zooplankton densities, we had annual measurements in Lake Aleknagik since 1963. Annual estimates of the growth and relative abundance of sockeye salmon juveniles and threespine stickleback (*Gasterosteus aculeatus*) were available for each of the Wood River lakes since 1958. Annual estimates of the abundance of sockeye salmon spawners and spawner-return statistics were available for each lake since 1946 and annual weather data have been collected for the region since 1919.

Our methods for measuring the effects of fertilization were to compare measurements between 1) fertilized and unfertilized areas of Little Togiak within a year, 2) fertilized and unfertilized years in Little Togiak, and 3) Little Togiak and Lake Aleknagik (and to some extent other lakes) in normal years compared to years of fertilizing. The lakes are usually

icefree from early June to mid-November but field observations were largely restricted to the period from mid-June to mid-September because most of the workers were university students who had to return for classes each autumn.

Some morphometric data and statistics on the adult sockeye salmon populations in Little Togiak and Lake Aleknagik are given in Table 1. Little Togiak is a narrow lake and it has two basins, a shallow upper basin (1.7 km^2) with a maximum depth of only 25 m and a deeper lower basin with a maximum depth of 83 m. Lake Aleknagik has a single basin. Alder predominates along the shore of Little Togiak and spruce trees are absent, whereas spruce, birch, and alder surround most of Lake Aleknagik. The littoral zone is rather restricted in Little Togiak with typically steep gradients and a rock or gravel bottom. In contrast, Lake Aleknagik contains some rather extensive, shallow bays with mud bottom. The relative production of sockeye salmon is similar for the two lakes; however, the populations in Lake Aleknagik are exploited at a slightly higher rate (mean rate of exploitation 1950 to 1976 was 45 percent vs. 41 percent) because it has a higher proportion of older and larger fish and the gillnet fishery is selective for these larger fish.

Physical Parameters

Solar radiation was measured daily with a Belfort pryheliometer and lake level was measured at Lake Nerka near the outlet of Little Togiak Lake. Surface temperatures were measured almost daily. The following measurements were made weekly in Little Togiak Lake and monthly in Lake Aleknagik, unless otherwise noted. Temperature-depth profiles were made at three stations on each lake. Secchi depth was measured at six stations, and when fertilizer was being applied, the measurements were made every two or three days. Conductivity was measured at 5 and 15 m at two stations on each lake. Nitrate-nitrogen, NH_3+ organic nitrogen, and total soluble phosphorus were measured at 5, 15, and 20 m at two stations in Little Togiak Lake and one station in Lake Nerka just before and after periods of fertilization in 1976 to 1978.

Biological Parameters

The concentration of chlorophyll a was determined from 2-liter water samples collected in Van Dorn bottles from 1, 3, 5, 7, 10, 15, 20, 30, and 45 m at two stations on each lake. Water

Table 1. Morphometric data for Little Togiak Lake (59°03'N, 159°09'W) and Lake Aleknagik (59°20'N, 158°48'W), and statistics on their sockeye salmon populations (1950-1976).

	Little Togiak	Aleknagik
Elevation (m)	23	10
Lake area (km^2)	6	83
Mean depth (m)	29	44
Maximum depth (m)	83	110
Drainage (km^2)	86	3,108
Annual discharge ($m^3 \times 10^6$)	102	4,139
Sockeye escapement (thousands)		
Minimum	3	31
Mean	20	183
Maximum	55	493
Average age composition of spawners (%):		
1.2	50	33
1.3	35	56
2.2	12	6
2.3	3	5
Return per spawner:		
Geometric mean	2.3	2.1
Minimum	0.9	0.4
Maximum	11.0	10.1

was filtered through an AA Millipore filter (0.8μ, 45 mm diameter) which was later dissolved in 90 percent acetone. Absorbances were measured on a Bausch & Lomb Spectronic 20 Spectrophotometer with a 1 cm path length. Calculations of chlorophyll *a* densities were made using formulas by Parsons and Strickland (1963). Phytoplankton species composition, numbers, and cell volumes were also calculated in 1976 and 1977.

The relative abundance and settled volume of zooplankton were determined from vertical hauls by a 1/2-m net with a mesh size of 234μ. Hauls were made from 60 m or near the bottom at shallower stations. The net did not sample nauplii or most rotifers (only *Asplanchna* sp. was usually caught); however, the mesh size retained the sizes of organisms typically eaten by the pelagic fish.

The relative abundance of emergent chironomids was determined from catches in conical, clear plastic traps similar to those described by Sublette and Dendy (1959). These were suspended 1 m off the bottom at a depth of about 3 m. Five traps were located in Little Togiak Lake and three in the lower end of Lake Aleknagik. The catches were counted every two or three days during the summer. Chironomid larvae, and other benthic organisms, were sampled with an Ekman dredge (225 cm^2 opening) at each end of Little Togiak Lake from depths of 3, 5, 7, and 10 m. Samples were taken at additional deeper stations in 1974, but the density of benthic organisms was low at sites deeper than 20 m. Sampling was usually conducted every other week between 20 June and 10 September.

The relative abundances and sizes of fish in the littoral zone were estimated from weekly beach seine hauls during late June to early August. Six stations were sampled in Little Togiak and ten in Lake Aleknagik. Juvenile sockeye and most of the threespine stickleback move offshore during late July. The fish were then sampled with a townet during mid-August to early September. Nine hauls of five minute duration were made on two nights in Little Togiak Lake and 12 hauls were made in Lake Aleknagik on one night each year. Stomach contents of fish sampled in Little Togiak Lake were examined in 1975 and 1976 to determine the composition and amount of food eaten.

The abundance, length, and weight of sockeye salmon smolts migrating from Little Togiak Lake were estimated from fyke net catches in Little Togiak River. The opening between the wings of the net was from 1/6 to 1/8 of the width of the river, depending on the flow. The net with a live box was fished almost continuously during June and July, and daily samples of 25 to 50 fish were collected to determine the age composition and size of the smolts. The Alaska Department of Fish and Game sampled the smolt migration from the lake system at the outlet of Lake Aleknagik and provided us with their data.

Arctic char (*Salvelinus alpinus*), which concentrate around the interconnecting rivers of the lake system in June and July to feed on migrating smolts, are the most abundant predators on juvenile sockeye in the lake system. We have sampled char in Little Togiak River by hook and line since 1972 to determine the number and occurrence of smolts in their stomachs.

Yearly, the Alaska Department of Fish and Game (ADF&G) utilized counting towers on Wood River to estimate the escapements of adult salmon into the lake system and aerial surveys to estimate the abundance of adult salmon in each of the lakes (by Mr. Mike Nelson). Since 1975, we have also counted the salmon as they migrated into Little Togiak Lake. These counts were made for 30 minutes out of each daylight hour and were later expanded to estimate the total escapement. The age composition in the escapement to Little Togiak Lake was determined from otoliths collected on the spawning grounds, and the runs (catch plus escapement) by age group to the lake were estimated from the ratios of run to escapement for the Wood River lake system.

Fertilization

A commercial diammonium phosphate fertilizer was used in 1974 and 1975 and a technical grade of diammonium phosphate was used in 1976 through 1978. Fertilizer was

dissolved in a drum and then sprayed on the surface of the lake from a moving boat. For purposes of sampling and the application of fertilizer, the lake was divided into three areas. Area A was the upper basin (1.7 km²), and fertilizer was applied over the upper half of this basin in 1974 and 1975 and over the entire basin in 1976 (Figure 2 and Table 2). Area B was the middle portion of the lake (1.8 km²), and it was fertilized in 1976 to 1978. Area C was the lower portion of the lake (2.5 km²), and it was fertilized in the same years; however, fertilizer was not applied near the lake outlet to minimize loss through the river. The amounts of phosphorus added to the lake were comparable to amounts contained in the salmon carcasses (Table 3). However, since most of the salmon decompose after mid-September and the decomposition is rather slow in the lake, nutrients from salmon carcasses probably did not affect primary production during the summer, but may have affected production in the fall or following spring.

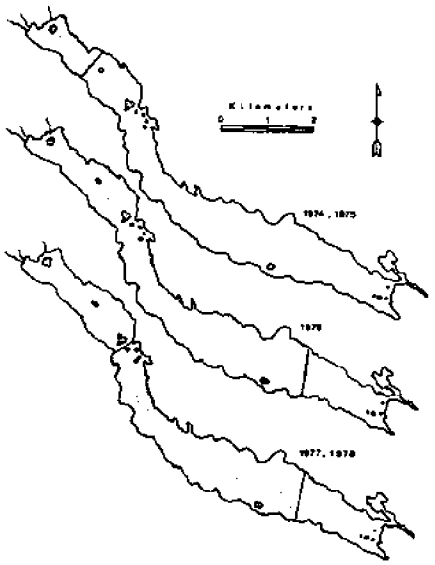

Figure 2. Areas fertilized (shaded) in Little Togiak Lake.

Results

Physical Parameters

During the years of our experiment there were some rather drastic variations in the environment that have made it difficult to interpret the biological effects of fertilization. Winter temperatures can affect the survival of eggs in the gravel, while spring and summer weather determines the length of the growing season and influences the growth rate of the fish. There were exceptionally cold winters (November to March) in Bristol Bay from 1969-70 through 1975-76; but the winter of 1976-77 was the mildest on record since 1919 (the Wood River lakes did not freeze over until March). The following winters of 1977-78 and 1978-79 were much milder than normal. Air temperatures in April and May largely determine when ice breakup occurs on the lakes. The date of ice breakup was about normal in 1973, one to two weeks late in 1975, 1976, and 1977, and one to two weeks early in 1974, 1978, and 1979.

Water temperatures during the summer in the upper 20 m were warmest in 1974; however,

Table 2. Schedules of applications of diammonium phosphate to Little Togiak Lake, 1974-1978.

Year	Date	Amount added (kg) by lake area A	B	C	Total	Density of applications (kg/km²)
1974	8/13	123			123	246
	14	88			88	176
	17	88			88	176
	18	80			80	160
	22	71			71	142
	23	106			106	212
	28	48			48	96
	29	57			57	114
Total/mean		661			661	165
1975	8/13	88			88	88
	17	66			66	66
	20	66			66	66
Total/mean		220			220	73
1976	7/12	133	89		222	85
	13	45	89	89	223	89
	14		44	178	222	92
	18	133	89		222	85
	19	89	89	44	222	89
	21			223	223	149
Total/mean		400	400	534	1,334	98
1977	7/13		312		312	173
	14			356	356	237
	20		222	89	311	111
	21		89	89	178	94
	22			177	177	118
Total/mean			623	711	1,334	147
1978	7/12			178	178	118
	13		178		178	99
	17			178	178	118
	18		178		178	99
	24			178	178	118
	25		178		178	99
	31			178	178	118
	8/1		178		178	99
	7		178		178	99
	8			178	178	118
Total/mean			890	890	1,780	109

the highest surface temperatures occurred in 1978 and the temperature in the entire water column was highest in 1979 (Figure 3). A thermocline develops in August usually between 10 and 15 m, but in 1979 the thermocline was near 20 m. Each year, water temperatures were about one degree warmer in Lake Aleknagik than in Little Togiak Lake.

Lake level declines during the winter to a low in mid-April and increases rapidly in May to a peak in late June as a result of the melting snow pack. Fluctuations during the remainder of the summer are caused by precipitation, which is usually highest in August and September. Lake level was exceptionally high during the summer of 1977 (Figure 3).

When fertilizer was applied in late August of 1974 and 1975, water temperatures were higher, but solar radiation and lake level were lower than when fertilizer was applied in late

Table 3. Amounts of phosphorus and nitrogen added to Little Togiak Lake annually from 1972 to 1979 in salmon carcasses in the fall and in diammonium phosphate fertilizer.

Year	Salmon escapement	Amount (kg) in salmon carcasses[1] P	N	Fertilizer added (kg) P	N	Dates
1972	14,000	126	1,222	0	0	
1973	14,000	149	1,445	0	0	
1974	48,000	413	4,017	155	140	August 13-29
1975	30,000	261	2,540	52	47	August 13-20
1976	18,000	150	1,460	313	283	July 12-21
1977	26,000	247	2,397	313	283	July 13-22
1978	45,000	384	3,731	418	377	July 12—August 8
1979	44,000	375	3,648	0	0	

[1]Assumes a weight of 4.5 lb (2.04 kg) for age .2 and 6.8 lb (3.08 kg) for age .3, and that a salmon contains 0.36% P and 3.5% N (Donaldson, 1967; Nelson and Edmondson, 1955).

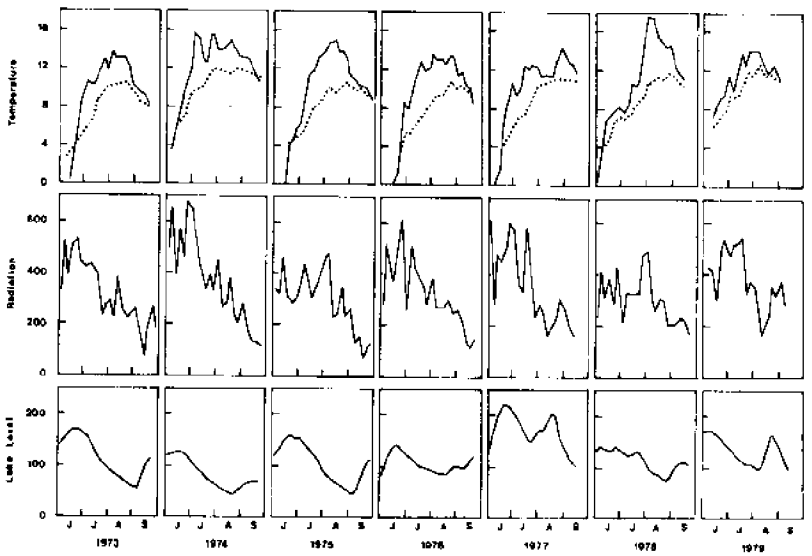

Figure 3. Average water temperature (C) at the surface (solid line) and 0-20 m (dotted line), and 5-day means of daily solar radiation (gm cal/cm²); and relative lake level (cm) during summers of 1973-1979.

July from 1976 to 1978 (Figure 3). In every year, except 1978, there were relatively heavy rains and overcast skies soon after the applications.

Conductivity did not vary greatly during the summer, except in 1977 when some rather extreme fluctuations occurred in Little Togiak Lake and to a lesser extent in Lake Aleknagik (Figure 4). These fluctuations were not closely correlated with fluctuations in other measurements except that the low values in July corresponded to exceptionally low concentrations of nitrogen whereas the high values in late August followed a heavy rainfall.

Variation in Secchi depth generally corresponded to variation in chlorophyll concentration except for the low values in late September 1973 and late August to early September 1979 (Figure 4). Runoff from rain caused a milky-white color on those occasions rather than the usual blue or green water color. In early August 1977, runoff plus a high phytoplankton density caused a milky-green color. When fertilizer was applied to only the upper basin of the lake (1974-75) Secchi depth decreased there but not in the lower basin. Secchi depth decreased from 11.5 m to 4 m within a few days after the addition of fertilizer to the upper basin in 1974.

Phosphorus concentrations averaged 10 to 14 µg/l prior to fertilization in 1976, 1977 and

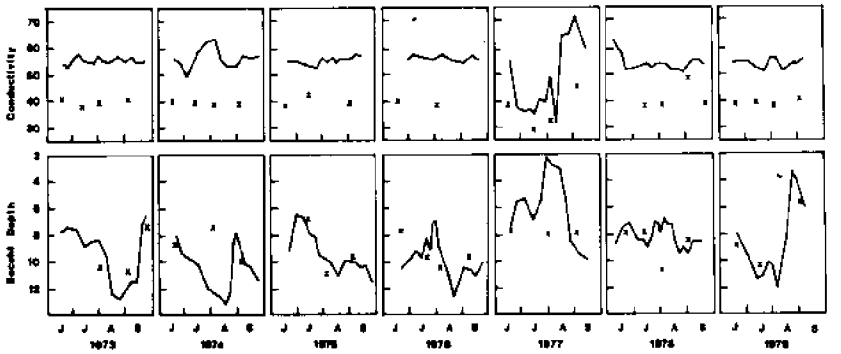

Figure 4. Conductivity (micromhos/cm) and Secchi depth (m) in Little Togiak Lake and Lake Aleknagik (x) during the summers of 1973-1979.

1978. The concentrations increased twofold in 1976 and 1978, and threefold in 1977 following fertilization. Total nitrogen did not change significantly in 1977 after fertilization. Mean values ranged from 111 to 163 μg/l. Total nitrogen declined from 236 before fertilization to 188 μg/l after fertilization in 1976 and from 400 to 270 μg/l in 1978. Nitrogen to phosphorus atomic ratios were usually greater than 20:1 except following the fertilization in 1977 when a low average ratio of 9:1 occurred in early August.

Phytoplankton

Primary production was not measured during our experiment in Little Togiak Lake for budgetary reasons. However, the rate of carbon-fixation was measured annually in Lake Aleknagik during the 1960s and in one year in Little Togiak Lake. No significant difference in the rates was observed between the lakes. In Lake Aleknagik, primary production was quite low below 20 m and usually zero below 30 m. Maximum rates of production nearly always occurred between 3 and 10 m and midday production rates for the upper 20 m averaged 14, 13, 12 and 15 $mgC/m^2/h$ in late June, mid-July, early August and early September, respectively. In our experiment, we relied on changes in chlorophyll *a* and measurements of zooplankton standing stock to evaluate the effect of fertilization on primary production.

Chlorophyll *a* increased in each year of fertilization, usually in the upper 10 m, and the amount of increase was approximately proportional to the density of phosphorus added and the amount of zooplankton present (Figure 5). In 1974 and 1975, chlorophyll increased only in the upper basin where the fertilizer was applied; however, in 1977 and 1978, when fertilizer was applied only in the lower basin, there were greater increases in the upper basin. This was probably caused by the movement of warm surface water toward the upper end of the lake and the lower density of zooplankton there. Most of the water comes into the lake at the west end from melting snow and the prevailing winds are easterly.

After the beginning of fertilization in August 1974 the chlorophyll concentration was significantly higher in Little Togiak Lake than in Lake Aleknagik until 1979 when fertilization was discontinued (Figure 6). The peak in chlorophyll concentration during late June to early July was related to the estimated amount of phosphorus in salmon carcasses in the previous fall. Excluding observations in 1979 (when the peak obviously occurred prior to our first measurements), but including observations in 1980, the correlation was .88 ($n = 7$). There was thus some evidence that nutrients from salmon carcasses have a positive effect on primary production during the early growth period of the progeny.

The phytoplankton communities were dominated by diatoms throughout the summers of 1976 and 1977. The greatest increase in numbers after fertilization occurred in the order

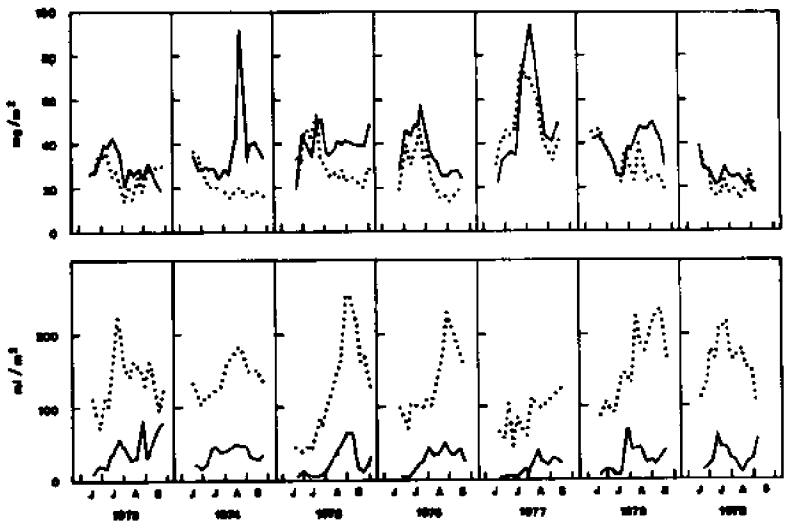

Figure 5. Amounts of chlorophyll a 0-20 m (top) and volumes of zooplankton (bottom) in the upper basin (solid line) and lower basin (dotted line) of Little Togiak Lake during summer 1973-1979.

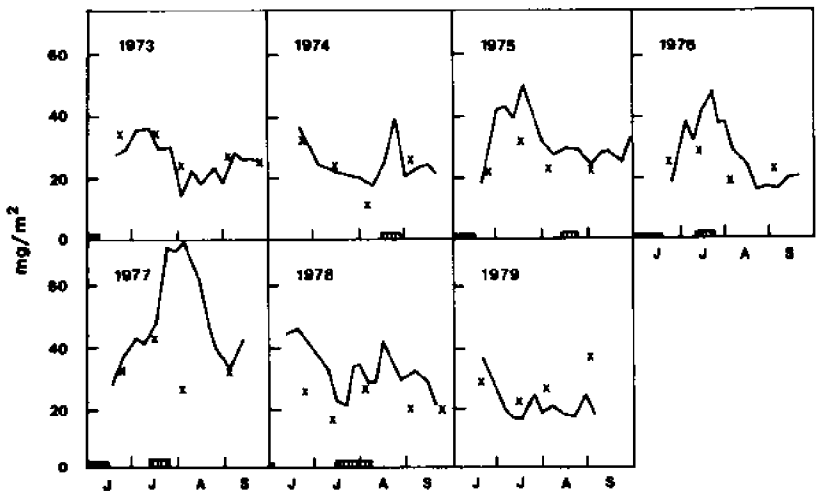

Figure 6. Amount of chlorophyll a (mg/m²) in the upper 20 m in Little Togiak Lake and Lake Aleknagik (X), 1973-1979. The solid bars indicate ice cover on Little Togiak Lake and the hatched bars indicate when fertilizer was applied.

Centrales in 1976, and in Pennales in 1977. There were increases in cell volumes of both orders in 1976, but only in the Pennales in 1977. *Asterionella formosa*, which contributed 23 percent of the total cell volume in 1976 and 80 percent in 1977, was the species most affected by fertilization. Following fertilization in 1977, 96.5 percent of the phytoplankton standing crop was *A. formosa*. *Cyclotella ocellata* was the next principal species affected by fertilization and in both years there were significant increases in the volume of this species following fertilization. Blue-green algae were absent in 1976, but *Anabaena* and *Chroococcus* did appear after fertilization in 1977.

Zooplankton

The volume of zooplankton early in the summer was generally higher in Lake Aleknagik

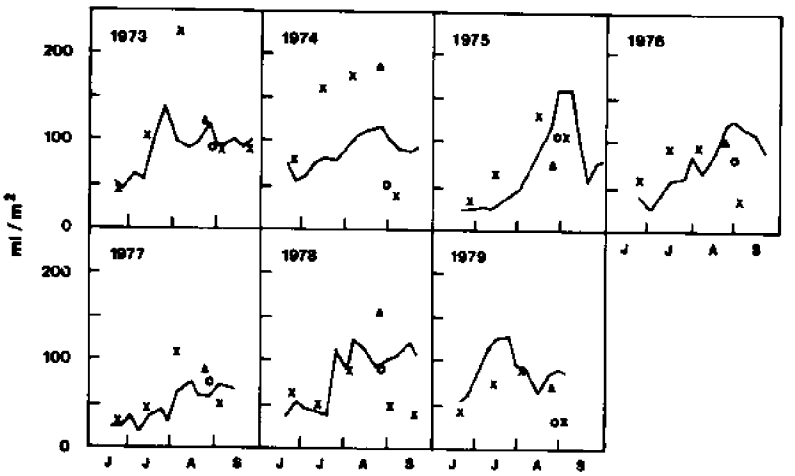

Figure 7. Settled volume of zooplankton (ml/m²) in Little Togiak Lake at weekly intervals and in lakes Aleknagik (X), Nerka (O), and Beverley and Kulik (▲).

than in Little Togiak Lake, except in 1979; late in the summer, the volume of zooplankton in Little Togiak Lake tended to be higher (Figure 7). The abundance of sockeye fry and threespine stickleback, which feed upon several species of zooplankton, was low in both lakes during 1973 and 1974, but high between 1975 and 1979. Even so, settled volumes of zooplankton were quite high during late August of 1975, and relative to the density of predators in the lake, again in late August of 1976. However, these high volumes were caused in part by relatively high densities of the large cladoceran *Holopedium gibberum* which was not commonly eaten by either fish species. *Holopedium* was scarce in 1973 and 1974, relatively abundant in late August of 1975 and 1976 (30,000 and 20,000/m², respectively) and then was nearly absent from Little Togiak Lake between 1977 and 1979.

We could not fully evaluate the effect of fertilization on the zooplankton community because our sampling terminated in September. This was often at a time when some species, such as *Diaptomus gracilis*, *Eurytemora yukonensis*, and *Daphnia longiremis* were increasing in abundance (Figure 8). Only the seasonal occurrence of *Eubosmina longispina* was typically within our sampling period. The densities of *Cyclops columbianus*, which can live longer than a year, were similar in the two lakes prior to fertilization; but it was generally more abundant in Little Togiak Lake after fertilization and there were exceptionally high densities in July 1979. Calanoid copepods were unusually numerous in September 1978, *Eubosmina* in July 1978 and 1979, and *Daphnia* in August 1976 and 1978, even though fish densities were high from 1975 to 1979. The abundances of all zooplankters were low in 1977. Between 1974 and 1978, the rotifer *Asplanchna* sp. was relatively scarce with maximum abundances of only 20,000 to 55,000/m². However, in late July 1979, *Asplanchna* became very abundant, reaching densities of 187,000/m². At that time it was next to *Cyclops* in abundance.

Benthos

Chironomids can be a major source of food for juvenile sockeye salmon and threespine stickleback in June and July when the abundance of zooplankton is usually low. Average catches of emergent chironomids were consistently greater in Lake Aleknagik than in Little Togiak Lake (Figure 9), which is probably because Lake Aleknagik has more suitable habitat for chironomid production than does Little Togiak Lake. Catches in Lake Aleknagik during 1974 and perhaps in 1979 were low partly because sampling did not begin soon after ice

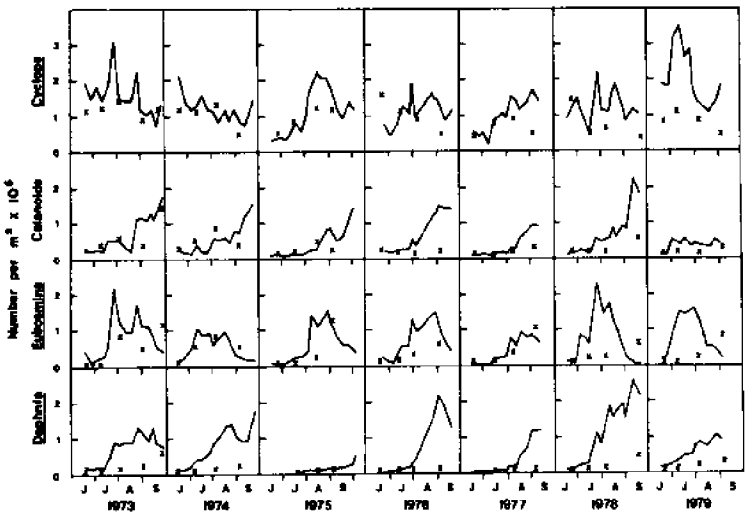

Figure 8. Summer abundance of zooplankters in Little Togiak Lake and Lake Aleknagik (X), 1973-1979.

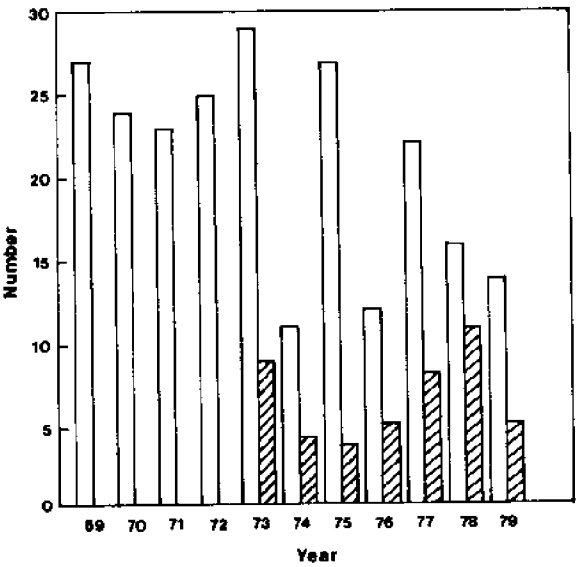

Figure 9. Average daily catches of emergent chironomids during the summer in Lake Aleknagik (1969-1979) and Little Togiak Lake (1973-1979).

breakup as in other years. In Little Togiak Lake, the highest emergence rates occurred during early August, whereas catches during June and early July were usually quite low (2 to 3 per trap per day). By contrast, there was often a high emergence rate in Lake Aleknagik soon after the ice was out in early June and then another peak emergence in late July. Neither the seasonal occurrence nor the average catch of chironomids in Little Togiak Lake were apparently affected by fertilization; however, fish abundance and presumably predation on chironomids were also higher after fertilization.

The relative abundance of chironomid larvae in benthic samples from Little Togiak Lake declined in August 1975 and then increased in 1978, but mean values for 1978 and 1979 were not significantly different from those in 1974 and 1975 (Figure 10). Differences in the relative

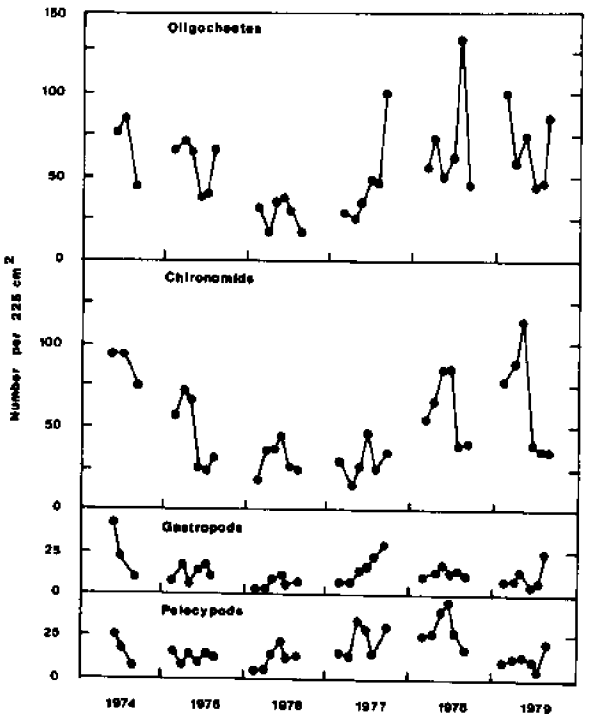

Figure 10. Relative abundances of benthic organisms in Little Togiak Lake. Each point is the geometric mean of eight observations. Early August to early September in 1974 and late June to early September in other years.

annual abundance of oligochaetes were similar to chironomids and the composition of benthic animals did not change significantly from 1974 through 1979. During 1977 and 1978, pea clams were more numerous than in prior years, but they were not abundant in 1979.

Fish Abundance and Growth

In both lakes, the average annual beach seine catches of sockeye salmon fry and threespine stickleback were lower in 1973 and 1974 than in 1975 to 1979. The increases in sockeye catches after 1974 correspond to increases in parent escapements to both lakes. Catches of threespine stickleback were relatively low throughout the lake system from 1972 through 1974 following exceptionally cold summers in 1971 and 1972. They began to increase in abundance during 1975. Catches of other species did not change significantly during the seven years. Beach seine catches for most species were higher in Lake Aleknagik than in Little Togiak Lake. Averages of the annual geometric mean catches were 102 and 92 sockeye salmon fry, 92 and 59 threespine stickleback, 17 and ten sculpin (*Cottus cognatus*), and ten and five ninespine stickleback (*Pungitius pungitius*), respectively. However, catches of Arctic char fry were significantly higher in Little Togiak Lake—19 compared with five in Lake Aleknagik.

The growth rates (mm/day between 20 June and 1 September) of sockeye salmon fry were higher in Lake Aleknagik, except in 1977 (.25 to .27) and 1979 (.28 to .39). The fry were longer on 1 September in Lake Aleknagik each year except 1979. Growth rates of age I threespine stickleback did not differ significantly between the lakes, but they were significantly longer in Lake Aleknagik. Growth rates of threespine stickleback and Arctic char were lower in both lakes during 1975 to 1979 when fish populations were denser than in 1973 and 1974.

Zooplankton abundance during the early summer and hence the amount of food in the fish

stomachs was lowest in the upper basin of Little Togiak Lake, where most of the sockeye spawn. Later in the summer, after the sockeye fry and threespine stickleback had moved offshore, there was little difference in the level of feeding between fish in the two basins of the lake. Stomachs of sockeye fry and threespine stickleback collected offshore throughout the lake during August usually contained 60 to 90 percent *Eubosmina*. Although *Cyclops* was more abundant in the zooplankton hauls, it occurs deeper in the water column than the cladocerans and probably is not as available to the fish. In August 1976, when *Daphnia* increased in the zooplankton, there was a corresponding increase in the stomachs of sockeye fry and threespine stickleback. However, fertilization did not greatly affect the growth of sockeye salmon fry up to 1 September, except perhaps in 1978 and 1979 (Figure 11). The average weights of fry in those years were heavier than expected from the density of parent spawners, but still within the range of variation from past years.

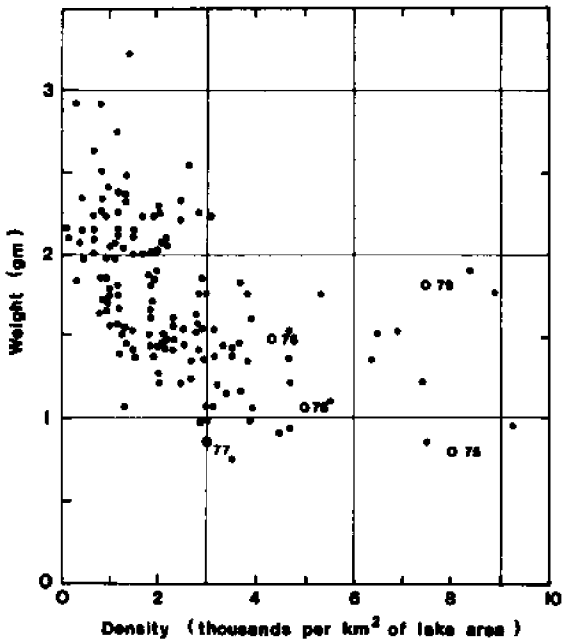

Figure 11. Mean weights of sockeye salmon fry on 1 September (1958-1979) in each of the Wood River lakes vs. the relative density of parent spawners (prior year). Observations from Little Togiak Lake following fertilization are indicated (o).

The mean lengths of age I smolts from Little Togiak Lake were comparable to those from the lake system prior to fertilization; however, the sampling was limited except in 1961 (Figure 12). After fertilization, smolts from Little Togiak Lake were significantly longer than smolts from the other lakes in 1977 and 1978.

Smolt migration from each lake in the system begins soon after each lake is clear of ice, and this happens progressively later from the lowest lake (Aleknagik) to the upper lake (Kulik), which is clear of ice about two weeks later than Lake Aleknagik. Therefore, the first smolts to migrate out of the system are almost entirely from Lake Aleknagik.

The mean weights of age I smolts early in the migrations from Lake Aleknagik were plotted against the mean weights of fry in Lake Aleknagik on 1 September in the previous year (Figure 13). If we assume that the correlation in the Lake Aleknagik data also applies to Little Togiak data then there were exceptional increases in weight from fry to smolts in Little Togiak following the fertilization. The observation for 1976 (the year of migration) is

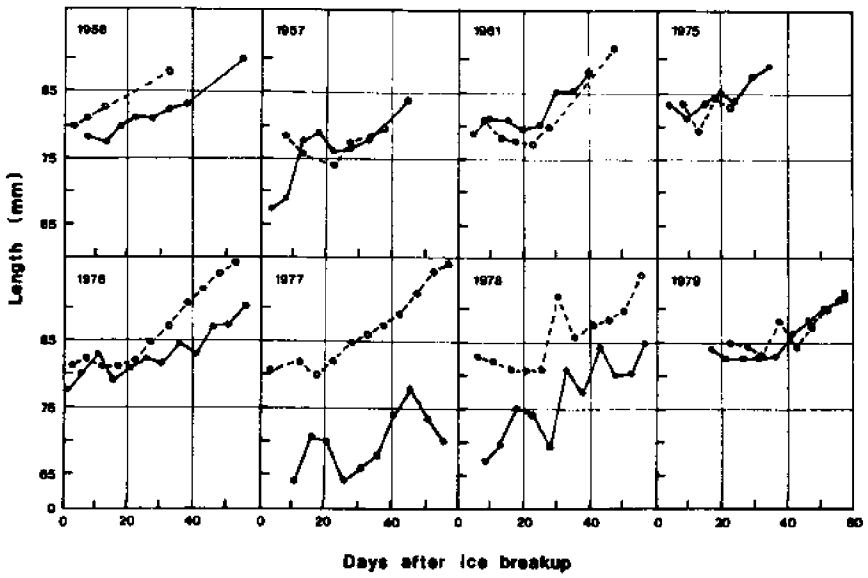

Figure 12. Mean lengths of age I smolts in migrations from Little Togiak (o) and the Wood River lakes at the outlet of Lake Aleknagik (•).

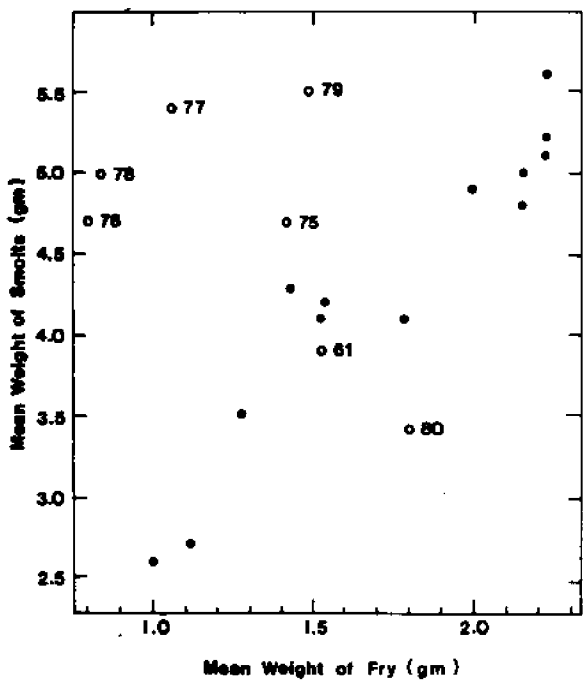

Figure 13. Mean weight of age I smolts during the first 20 days of migration (year shown) vs. the mean weight of fry on 1 September of the previous year in Lake Aleknagik (•) and Little Togiak Lake (o).

misleading because nearly as many age II smolts migrated in 1977 as age I smolts in 1976. The lake was not fertilized in 1979 and although the fry had exceptionally good growth up to 1 September, their growth during September/October was apparently very poor because the smolts that migrated in 1980 were the smallest ever observed from Little Togiak Lake.

The number of smolts in the migration from Little Togiak Lake were estimated during five years. Sampling began too late in 1979 to obtain a reliable estimate. The estimates (in thousands) were: 666 in 1961, 187 in 1976, 510 (28 percent age II) in 1977, 541 in 1978, and 1,033 in 1980. Thus, the largest migrations contained the smallest smolts.

The purpose of fertilization is to increase the survival of juveniles by either reducing mortality from malnutrition (starvation) or reducing mortality from predation, on the assumption that this mortality is a function of size or health of the juvenile salmon. Predation by Arctic char on sockeye salmon smolts in Little Togiak River apparently decreased following fertilization of the lake (Table 4). The number of smolts in char stomachs is partly a function of the size of the char; however from 1977 to 1979, the average numbers of smolts in char stomachs were low relative to the average length of the char sampled in those years. Of course predation by Arctic char in Little Togiak River is probably a minor source of mortality between juveniles in the lake and returning adult salmon, two to three years later.

Table 4. Statistics from stomach samples of Arctic char collected by hook and line from Little Togiak River during 30 days following ice breakup, 1972-1979.

	1972	1973	1974	1975	1976	1977	1978	1979
Samples collected in the day:								
Number examined	—	49	—	18	49	206	170	50
Mean length (mm)	—	470	—	449	435	399	443	431
Percent containing smolts	—	67	—	50	61	21	42	38
Mean number of smolts	—	4.9	—	3.3	3.1	0.5	1.6	1.6
Samples collected at night:								
Number examined	82	72	64	53	47	119	146	128
Mean length (mm)	446	429	429	404	400	411	431	441
Percent containing smolts	60	29	39	31	51	11	42	20
Mean number of smolts	4.5	1.6	1.6	1.3	1.2	0.3	1.4	1.0
Sockeye escapement to Little Togiak Lake in year-2 (thousands)	55	24	14	14	48	30	18	26
Mean length of smolts in migration (mm)	—	83	90	84	84	88	84	85
Lake level (cm)	177	161	121	150	122	206	133	164
Total number of char removed	463	208	283	71[1]	113[1]	574	587	344
Total number of char measured	729	208	263	71	113	574	587	344
Mean length (mm)	444	444	435	415	427	388	432	427

[1]Char were also removed by the Alaska Department of Fish and Game; however, the total number removed in each of these years was less than 200.

Adult Returns

The most difficult effects of fertilization to evaluate and yet the most important are the effects on the marine survival of sockeye salmon and the number of returning adults. Positive effects on primary and secondary productivity and the growth of juvenile sockeye salmon were demonstrated in the Bare Lake and Great Central Lake experiments (Nelson and Edmondson, 1955; LeBrasseur, et al., 1978). Effects on primary production were also demonstrated in four other British Columbia lakes in experiments currently in progress (Stockner, et al., 1980). However, effects on the adult returns to Bare Lake were not fully evaluated (Nelson, 1959) and the effects on the adult returns to Great Central Lake were difficult to ascertain because the runs also increased in an adjacent unfertilized lake and there was an increasing trend in the runs prior to fertilization.

The initial adult returns from juveniles in Little Togiak Lake when it was first fertilized came in 1977; however, first returns from the whole-lake fertilization came in 1979 and the final returns will not come until 1982. The runs to Little Togiak Lake were exceptionally large from 1978 to 1980 but so were the runs to the entire lake system (Figure 14). The runs or catches of all species of salmon in Bristol Bay and indeed most of Alaska have been large in

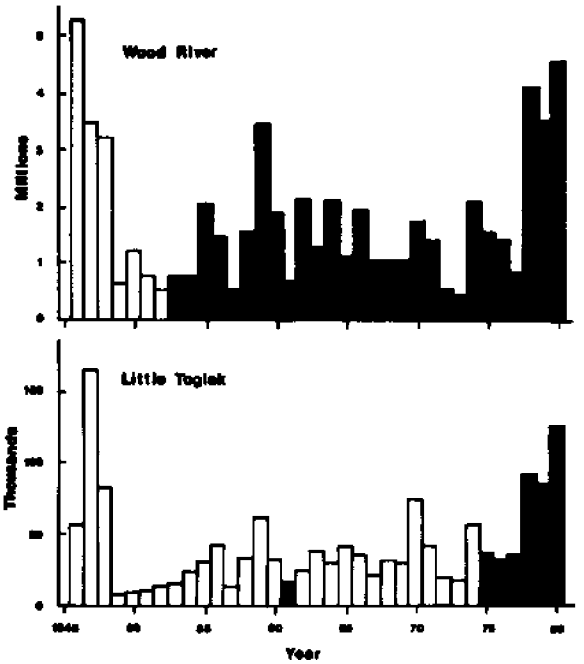

Figure 14. Annual runs of sockeye salmon (catch plus escapement) to the Wood River lake system and Little Togiak Lake. Open bars when escapements estimated by aerial survey and solid bars when estimated by counts on the river.

these recent years. So relative to the area-wide increase, the initial returns of adult sockeye salmon from the fertilization of Little Togiak Lake were not exceptional.

Summary

Little Togiak Lake was fertilized with diammonium phosphate to enhance the growth and survival of juvenile sockeye salmon, and thus increase the abundance of returning adult salmon. Fertilization increased primary production and increases in secondary production (food for juvenile sockeye) were observed in some cases but not in others. Growth of fish during the early summer was not significantly increased except perhaps in 1979 (the year following the last fertilization) when there was an exceptionally high density of *Cyclops*. The apparent growth from juveniles on 1 September to smolts the following spring was greatly increased after fertilization. Presumably the abundances of *Daphnia* and perhaps calanoid copepods were increased in the fall; however, direct observations were seldom made after mid-September. The age I sockeye salmon smolts were larger relative to the abundance of parent spawners, their size as fry, and the size of other smolts in the lake system. However, they were not larger than the largest smolts observed at Wood River in past years when abundance was low. The fertilization thus produced smolts that were above average in size during years in which they normally would have been well below average in size. Whether this larger size will result in higher marine survival and more returning adults has yet to be determined, since the early returns of adult salmon to Little Togiak Lake have not been exceptionally large relative to the returns to the other lakes in the system.

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Trophic Level of Lake Dalneye (Kamchatka) in the 1970s

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Observations on the conditions in Lake Dalneye have been conducted over four decades. The data obtained from this complex research program executed by E. M. Krokhin and F. V. Krogius revealed some patterns relevant to salmon lakes similar in morphometric and climatic conditions (Krogius, 1948 and 1956; Krokhin, 1957, 1960 and 1968a). Up to now the lake has undergone great changes. Fish migrations to the spawning grounds sharply decreased in the late 1940s. The abundance of spawning fish in the 1950s decreased from 60,000 to 70,000 individuals to 2,000 to 10,000 individuals per year (Krokhin, 1968b). This changed the ecological conditions which had been created in the spawning lakes over many years. Krokhin (1957 and 1959) proved that decomposition of spawned fish led to enrichment of lake water with phosphates. Since allochthonic (input) into Lake Dalneye is low, 25 percent of all phosphate input was produced by carcasses. When the annual numbers of spawners were less than 500 to 700 per 1 million m^3 of lake volume, their influence upon phosphate concentration decreased. Current observations show a similar pattern.

Lake Dalneye is a well investigated model reservoir, especially in ichthyological and hydrological aspects. The primary production level was regularly determined by indirect chlorophyll method. This method is simple but provides only an approximate level of planktonic photosynthesis. Oxygen determinations in areas of low algal production and great depths are subject to problems in methodology (Kurenkov, 1966; Romanenko, 1971). F. V. Krogius (1978) has measured primary production by plankton count and phosphate utilization in Lake Dalneye for four decades. The procedure is of value in reservoirs with high phosphorous concentration. The validity of this procedure is borne out by the work of Petersen (1977) using C_{14} to measure primary production.

The most precise counting of the abundance of organic matter newly formed during photosynthesis is possible when using radioactive carbon. Investigations were conducted in 1970/1971 and in 1979 under conditions of a further decrease in spawning migrations of red salmon. At the same time, total organic carbon was counted in suspensions of vegetation to reveal the trophic character of the reservoir during two seasons.

Materials and Methods

The research was conducted at a central station of Lake Dalneye at a depth of 56 m. Intervals between surveys in the seasonal cycle of observations were ten to 20 days depending on the change of biological phenomena in the lake. The samples were picked up from eight to ten depths, the positions of which were selected with temperature stratification taken into account. Water was taken with Suslyaev's plastic water bottle with a 2.5 liter capacity.

Phytoplankton production was counted with the help of the radioactive carbon method

(Steemann-Neillsen, 1952) modified by Yu. I. Sorokin (1958). Hydrocarbon content in water was measured by the V. I. Romanenko and S. I. Kuznetsov (1974) method. To count seston abundance in the lake the water was filtered through glasswool filters. The volume of the filtered water was in most cases about 1 liter. Both filters and sediment were acidified with bichromate (Ostapenya, 1965). On the basis of the layers' parameters of Lake Dalneye calculated by Krokhin (1973) in accordance with lake morphometry, the total abundance of seston and phytoplankton production per 1 m^2 of lake surface in the seasonal cycle of observations was counted. The sum of newly formed autochthonous organic matter in the lake was calculated following an integral curve. The average seasonal value of daily primary production and organic carbon suspensions was obtained by the same technique.

Plankton Primary Production

Spring blossoming of Lake Dalneye is caused by mass development of the diatom (*Stephanodiscus astraea* var. *minutulus* [Kiitz] Grun.), which comprises 90 percent of all the phytoplankton (Sorokin, et al., 1974). According to a white disc, water transparency decreases from 9 to 15 m in April down to 2 to 3 m at the period of maximum algae development. The basis of productivity of any aquatic ecosystem is the production of organic matter by phytoplankton. The trophogenic layer of Lake Dalneye, where this process occurs, is 20 m on the average, epi- and metalimnion included. Optimum conditions for phytoplankton photosynthesis are created in the layer of double transparency (in May and June at a depth of 5 to 7 m). In summer and fall absolute values of photosynthesis are very low and transparency increases up to 6 to 7 m. The highest primary production for this period is at this depth (Figure 1). Distribution of active algae potentially capable to reproduce has another character (curves K_p). They concentrate in the thermocline zone and following it go deeper during the vegetative season. As a result the huge algae mass suffers "lighting hunger." This phenomenon is typical of deep reservoirs. The curve rate of relative photosynthesis in the water (K_c) in June had maximum indices coinciding with the most favorable lighting in the depths. The second increase in primary production was observed at the depth of concentration of the main phytoplankton mass in July.

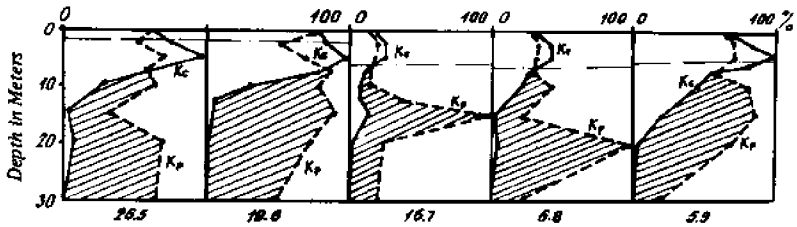

Figure 1. Distribution of algae active for potential photosynthesis (K_p, in % from the surface) and relative photosynthesis (K_c, in % from the surface) in trophogenic layer in different periods of vegetation. Hatched square area reflects the degree of "lighting hunger" of algae; horizontal dotted line means water transparency.

Fluctuations in phytoplankton production for the vegetation period of 1979 had a tendency similar to the 1970/1971 season. However, some differences were evident. Spring algae development started nearly a month earlier and reached a higher level with a maximum of 5.3 grS/m^2. It was 20 percent larger. The main autotrophic phase of the production process was observed during May and June 1979. This is common for reservoirs of low and medium trophic character. Summer and fall blossoming of Lake Dalneye has notably decreased as compared with the early 1970s (Figure 2). This has had a negative effect on food resources of zooplankton for that period. For the first month of the vegetation period the organic matter

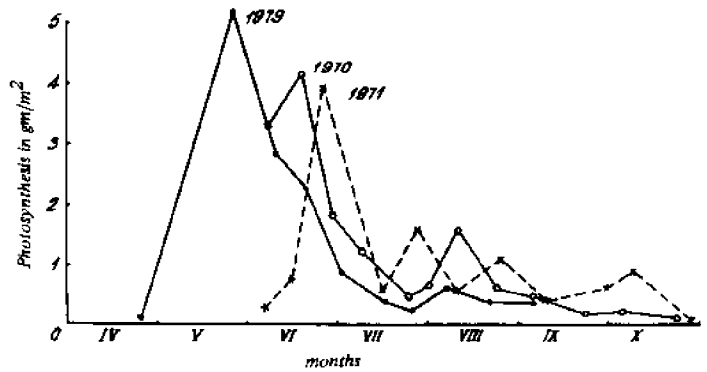

Figure 2. Seasonal fluctuations of primary production (grS/m²) in various observation periods.

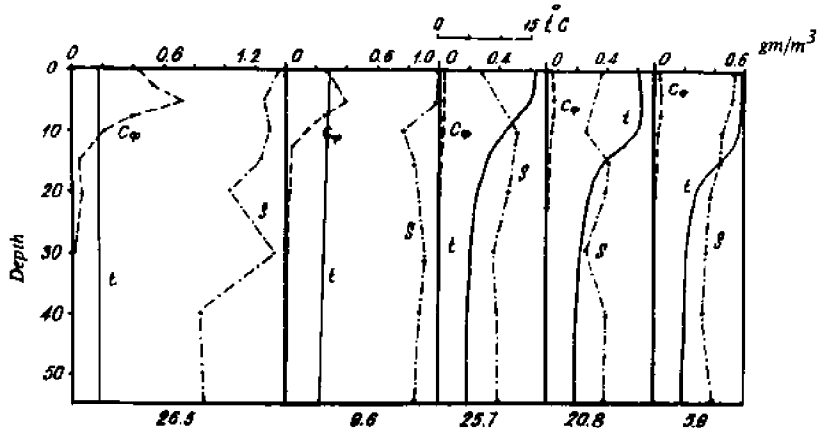

Figure 3. Vertical distribution of temperature (t), primary production (C_p, S/m³) and seston (S, grS/m³) during vegetation season.

per square meter of lake surface was ten times more than for the entire following period. A total of about 230 grS or 2160 kcal/m² was produced in the lake in 1979; that is 40 percent higher than in 1970/1971.

Seston

The abundance of organic matter of seston serves as a reliable index of reservoir productivity (Vinberg, et al., 1971). Vertical distribution of suspensions enables us to determine their density in some layers of lake water (Figure 3). In April the seston is evenly distributed in the depths under ice amounting to 0.3 grS/m³ early in the month and 0.2 grS/m³ at the end of the month. During spring stagnation its concentration in epilimnion, as a result of lake blossoming, reached 1.3 grS/m³ with 0.8 to 1.0 grS/m³ in the depths. Up to midsummer organic suspension in the layer 0 to 10 m contained 0.9 to 1.1 grS/m³. It is interesting to note that in the period from mid-June to mid-July seston abundance in trophogenic layers is twice that of hypolimnion layers. Afterwards, its concentration is distributed evenly through the entire water mass. This fact may serve as a indicator of the time of dying off of the spring phytoplankton population. General lowering of seston concentration in the lake at the end of the summer and in the fall testifies to the consumption of organic suspensions or detritus by zooplankton.

Seston abundance in the lake depends directly upon the photosynthesis intensity. When we follow the changes in total organic suspension and primary production content for the whole season it will be obvious that the largest part is caused by phytoplankton development. In spite of the differences in the percentage of synthesized matter from all seston during the summer, the average values for 1971 and 1979 are constant: 7.5 and 7.6 percent, respectively. This is an indicator of the stability of biological processes within the ecosystem, which may serve as a criterion for a reservoir type. Suspensions, according to their absolute values, are an order higher than the value of plankton photosynthesis. This is in agreement with correlation of production and algae biomass data (Sorokin, et al., 1974), as well as the contents of detritus of phytogenic origin in the seston of Lake Dalneye (Pavelieva, 1974) (see Figure 4). When comparing the total seston per average square meter of lake surface for two seasons, we noted that the trophic level in 1979, following this index, slightly increased (mean concentration was 18.3 grS/m²) and was in good sequence with the autochthonous phase of the production process. During domination of heterotrophic processes seston contents in both investigative years became equal (Figure 5).

Specificity of Determination of Trophic Level of Salmon Lakes of Lake Dalneye Type

The level of primary production is the basic criterion for characterizing the reservoir type (Vinberg, 1956 and 1961; Robinson and Barroclough, 1978). In this case, we first must decide

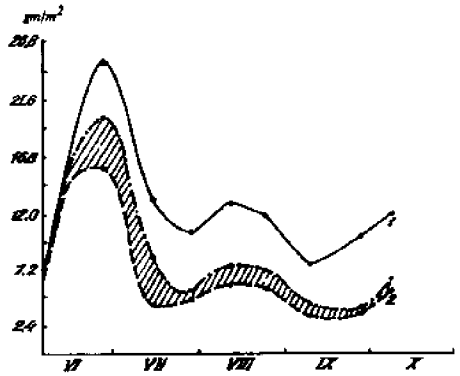

Figure 4. Seasonal pattern of fluctuations of abundance of seston (1), detritus (2) and phytoplankton (hatched).

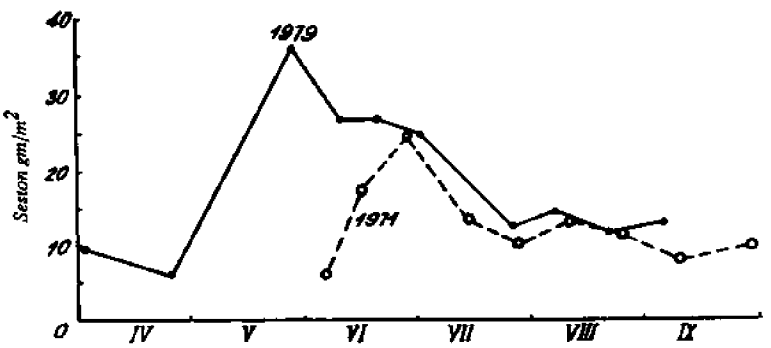

Figure 5. Seston dynamics (grS/m²) during two seasons of vegetation.

what value the investigators mean when determining the intensity of photosynthesis by assimilated $C^{14}O_2$. On the basis of some literature and my own data obtained from Lake Dalneye, we may consider the given values of organic matter newly formed during photosynthesis to be the net plankton production. When using a transitive coefficient of 1.54 (Romanenko, 1976) the gross primary production comprised 2300 kcal/m² in the early 1970s, with 3300 kcal/m² in 1979, a 44 percent increase. According to the primary production value per square meter, Lake Dalneye ought to be called a medium trophic reservoir, as E. M. Krokhin (1968a) suggested. But to characterize a reservoir we should take into account the indices of primary production (as well as other characteristics) per unit volume. This correlates with the fact that in oligotrophic reservoirs with good transparency, photosynthesis also occurs at considerable depths showing values per square meter relevant to shallow mesotrophic or even eutrophic reservoirs (Vinberg, 1960; Romanenko, 1965). Fluctuation of the net plankton production from 5 to 700 grS/m³ was observed in the trophogenic water layer of Lake Dalneye during three vegetative seasons. The first figure corresponds to the lower values for oligotrophic reservoirs. The second one refers to the upper values for mesotrophic lakes (Vinberg, et al., 1971; Kuznetsov, et al., 1971). This kind of fluctuation is observed from the moment of spring homothermal conditions to the cessation of lake blossoming. As a result of photosynthesis 250 to 350 grS/m² of the seston is formed in the lake during the ice-free period.

At present, most investigators (Halmann, 1972; Powers, et al., 1972; Tanner, et al., 1972; Welch, et al., 1978) have reached the conclusion that phosphorus is the basic element affecting the change of primary production in a reservoir, especially one of low trophic character. Carcass decomposition in Lake Dalneye in the 1970s did not considerably influence the phosphorus regime because its abundance sharply decreased (Krokhin, 1967). Hence, nutrient rotation within the ecosystem was exercised with the help of organic matter synthesis by phytoplankton, and with further dissimulation through the detritus and zooplanktonic area. Ecosystem losses of organic phosphorus compounds, as a part of downstream migrant juveniles of red salmon and seston removal from the lake, are to some extent indemnified by carcass decomposition together with ground and rain waters and brooks flowing into the lake.

Determination of the seasonal pattern of primary production of organic matter of Lake Dalneye in 1970/1971 and 1979 shows that its level has stabilized and at high values. This occurs because of exclusively favorable conditions for using even very small nutrient concentrations in early summer. Phytoplankton production in late summer keeps the level relevant to oligotrophic reservoirs, i.e., 0.2 to 0.7 grS/m² (Kuznetsov, et al., 1971; Sorokin and Fedorov, 1968). At present, the effect of relative lake oligotrophization is shown in the weakening of its summer and fall blossoming (coinciding with the extreme lack of mineral phosphorus). Quite an abundant reserve of organic matter is formed in the lake during the vegetative season.

To take into account the direct influence of primary production of a growing reservoir upon salmon production (R. C. Dugdale and V. A. Dugdale, 1961; Manzer, 1976; Nelson and Edmondson, 1955), it would be advisable to direct fisheries and cultural works to fertilize salmon lakes in our country. However, it is necessary to take the specific character of every reservoir into account. For this purpose, integrated works to study the ecosystem as a whole are needed. They should consist both of hydrological and abiotic factors affecting salmon production.

Conclusions

The phytoplankton community of Lake Dalneye develops under spring homothermal conditions with maximal concentrations of nutrient compounds for the vegetative season. Dur-

ing stratification, N and P abundance in the trophogenic layer is depleted. This leads to a decrease in primary production.

The main portion of the active algae in the meta- and hypolimnion lacks subwater lighting during the whole productive period.

Fluctuations in primary production for all the investigated vegetation periods were similar. In 1979, the spring blossoming of the lake was observed a month earlier and reached a higher level—5.3 grS/m². This is 20 percent larger than in 1970/1971. Synthesis of organic matter in summer and fall was, as a whole, lower than in the early 1970s.

The total content of organic suspension in the lake was a result of primary plankton production. Suspensions, according to their absolute values, were nearly an order higher than the value of daily photosynthesis per square meter.

According to suspension abundance and phytoplankton production, the trophic level of Lake Dalneye in 1979 increased slightly in comparison to the 1970/1971 level. Correlation of these values for these years is constant. This proves the stability of biological processes within the ecosystem.

Gross primary production of plankton (when using the transitive coefficient of 1.54) comprised in 1970/1971 an average of 2300 kcal/m², with 3300 kcal/m² in 1979.

At present, the trophic level of Lake Dalneye is expressed by rather high values. However, primary production in summer and fall is very low; zooplankton exists due to detritus. This leads to energy loss within the ecosystem. In this sense we may speak of the relative oligotrophization of Lake Dalneye in recent years.

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A Progress Report on the Effect of Rearing Density on Subsequent Survival of Capilano Coho

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Abstract

Two experiments were conducted at Capilano Salmon Hatchery, North Vancouver, B.C. to determine the effect of rearing density on subsequent survival to adult. The first experiment involved 1975 brood coho transferred as eyed eggs from the Big Qualicum River. The second experiment utilized 1977 brood coho native to Capilano. Both groups of eggs were incubated in Heath trays (~ 7,500 eggs/tray) until March 1976 and 1978 respectively, at which time the fry were moved to 6.5m long fiberglass troughs to commence rearing. The loading rate in the troughs was ~ 35,000 fry/m³. The fry were fed Oregon Moist Pellets (OMP) based on the standard feeding schedule and by mid-June had reached an average size of 1.5 to 3.0 g. The fry were then loaded into a Burrows pond (24.6m x 5.6m x 0.9m) at four different density levels (flow rate in all ponds was 28 l/sec).

Standard hatchery rearing practices were followed and all groups were maintained on OMP diet. Prior to release, fish from each of the four groups were marked by removal of the adipose fin and a binary coded wire tag was injected into the snout. Once the fish were marked they were returned to the pond from which they had been taken. From the total number of marked fish in each pond (23 to 34 percent of each group were marked) a new population estimate was made using the marked to unmarked ratio.

During rearing, mortalities were recorded in each of the ponds and ranged from 1.0 to 3.4 percent. However, the population estimates based on the marked to unmarked ratios revealed discrepancy losses of 11.3 to 18.6 percent. As it was impossible to determine at which point these losses occurred, it was assumed that the number of fish in the pond at release was the calculated number, and for purposes of comparison between groups, this number was used to express the density of fish reared:

Density	Low	Med. Low	Med. High	High	
1975 brood	500	664	731	901	fish/m²
1977 brood	504	675	751	931	fish/m²

Both broods were released on the evenings of 6 and 7 June in 1977 and 1979 respectively at an average size of:

Density	Low	Med. Low	Med. High	High
1975 brood	15.6g	19.0g	19.1g	14.4g
1977 brood	15.3g	17.6g	16.3g	14.7g

To compare the relative survival between the different density groups it was decided to use only the number of marks observed in the fishery and escapement and not the expanded values adjusted for sampling rates in specific fisheries. Recovery data for the 1975 brood is complete while less than 10 percent of the marked recoveries for the 1977 brood have been reported to date. For purposes of comparison within a brood year the groups with the lowest survival rate have arbitrarily been assigned a value of 1. The survival rates of the other groups within that brood year are relative.

Density	Low	Med. Low	Med. High	High
1975 brood	1.84	1.42	1.35	1.00
1977 brood	1.80	1.73	1.16	1.00

For the range of densities tested, it is clear that a pond with half as many fish (low density) will produce just as many adult coho to the fishery and escapement as will a pond of fish reared at high density.

Section IV
Effects of Environmental Stress on Fish Quality and Survival

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Effects of Environmental Stressors in Aquacultural Systems on Quality, Smoltification and Early Marine Survival of Anadromous Fish

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Abstract

Fish in intensive culture are affected both by the demands of the aquatic environment itself and by the stress of hatchery conditions and practices such as handling, crowding, transporting, and disease treatments. In anadromous fish, the effects of stress include inhibition of the parr-smolt transformation, impaired migratory behavior, and reduced early marine survival. The physiological tolerance of fish to stress is discussed and methods are presented for its quantitative assessment, which can be used to monitor impacts on fish health and quality during rearing. Recommendations are given for the environmental conditions and hatchery practices that will reduce environmental stress and increase efficiency in the use of artificial propagation for the enhancement of fisheries.

Environmental Stress: An Overview and Definition

In a discussion of the physiology of fish in intensive aquaculture, a good starting place is a consideration of the effect of normal conditions in the aquatic environment itself. Fishes commonly live under somewhat rigorous chemical and physical conditions, many of which have no analogue in the terrestrial environment. They are physiologically adapted to these conditions, but this adaptation does not imply the absence of an energy drain (Lugo, 1978). First, fish must expend a significant number of calories (from the diet) in overcoming frictional drag during swimming and in moving water over the gills to obtain oxygen. Second, the water acts as a dialyzing medium for the blood. Thus, the epinephrine-induced increases in gill circulation when additional oxygen is needed result, as well, in an increased water influx. The electrolyte loss caused by the resulting diuresis can be severe. Third, the amount of dissolved oxygen available for respiration is strictly limited; it is normally only about 10 to 12 mg/l, is frequently much less, and can fluctuate widely. This restricted availability of oxygen is in contrast to the relatively steady 300 mg/l available to air breathers.

Thus, homeostatic control in fishes is continually challenged by the normal physiochemical demands of the aquatic environment itself. Added to this may be stress from habitat alterations, interspecific competition from other fishes, and (in hatcheries) stress from operating procedures.

Fish in hatcheries are routinely subjected to relatively intensive biological, physical, and chemical conditions. These include high population densities, handling, transporting, disease treatments, and fluctuating water temperatures and chemistry (Piper, 1981). All of these conditions, together with the fright that accompanies them, can impose a considerable load, or stress, on the physiological systems of fish. Stress that exceeds a fish's physiological

ability to tolerate is, of course, eventually lethal. Individual stress factors in aquaculture are usually sublethal in themselves; but since they frequently occur in multiples, the resulting physiological load imposed can reduce growth, predispose to physiological, or infectious diseases (if pathogens are present), and impair the ability of fish to survive additional stress following release.

The present use of the term "stress" is inconsistent. It is loosely taken to mean both the physiological response of the animal, and the environmental stress factor itself. At the organism level, stress was originally defined as "the sum of all the physiological responses by which an animal tries to maintain or re-establish a normal metabolism in the face of a physical or chemical force" (Selye, 1950). More recently, stress has been considered to be the effect of any environmental alteration or challenge that extends homeostatic or other stabilizing processes beyond normal limits, at any level of biological organization—species, population, or ecosystem (Esch and Hazen, 1978; Peters, 1979; Wedemeyer and McLeay, 1980). The present recommendation is to use "stressor" to mean the environmental factor (although terms such as "handling stress" are frequently used). Thus, a stress factor, or stressor, is an environmental or biological challenge that is severe enough to require a physiological or behavioral response by a fish. If the stress response can reestablish a satisfactory relationship between the changed environment and the fish, adaptation to the stressor will occur. Such an adaptation requires that a series of physiological changes take place, which in higher animals has been called the general adaptation syndrome.

In fishes, the stress response involves a series of primary, secondary, and tertiary physiological changes that are recognizably similar for any stressing agent, e.g., handling, disease treatments, fright, forced swimming, anesthesia, rapid temperature changes, and scale loss. Thus, the stress response of fishes is at least similar to that occurring in the higher vertebrates (Gronow, 1974).

The sequence of the physiological changes, as fish attempt to maintain homeostasis in the face of a stressful biological or environmental challenge can be outlined as follows (Chavin, 1973; Donaldson and Dye, 1975; Schreck, et al., 1976; Mazeaud, et al., 1977; Peters, 1979; Wedemeyer and McLeay, 1980).

Primary (endocrine) effects
- Adrenocorticotrophic hormone is released from the adenohypophysis
- Catecholamines and corticosteroid hormones are released from the interrenal

Secondary (hormone induced) effects
- Blood chemistry and hematological changes, including hyperglycemia, hyperlacticemia, hypochloremia, and leucopenia
- Tissue changes, such as depletion of liver glycogen and interrenal vitamin C, and interrenal hypertrophy
- Diuresis occurs resulting in osmoregulatory dysfunctions due to blood electrolyte loss via the urine

Tertiary effects
- Physiological and behavioral disorders
  - Delayed mortality due to blood electrolyte imbalances
  - Reduced growth and food conversion
  - Inhibition of the parr-smolt transformation of anadromous fish
  - Increased susceptibility to fish diseases due to impaired phagoeytosis, immune, and inflammatory responses
- Population and ecosystem effects
  - Reduced intrinsic growth rate, recruitment, compensatory reserve
  - Altered species abundance and diversity.

The duration of the stress has an important bearing on the physiological outcome. For example, an acute stress results in a partial depletion of vitamin C, corticosteroids, and

cholesterol in the interrenal tissue and anterior pituitary. Under chronic stress, a complete depletion will occur together with tissue hyperplasia and hypertrophy.

Thus, a fish's survival in the face of environmental stressors depends upon its ability to regulate compensatory processes sufficiently to maintain the degree of homeostasis required for life. A stressor requiring an adjustment that exceeds the fish's capacity for accommodation eventually results in disease or is lethal. An understanding of the physiology of the stress response, and the degree of change to which fish can adapt through this mechanism, is important to a definition of the aquacultural conditions required to maintain optimum health. Although fishes can usually survive environmental stress for limited periods because of their homeostatic capabilities, this should never be used as an excuse for operating aquacultural facilities under marginal conditions. Instead, these capabilities should be used to set priorities and limits for the environmental conditions needed to optimize fish health, smolt functionality, and marine survival.

The Stress of Intensive Fish Culture

Fish in aquacultural facilities are continuously under some degree of stress because of required hatchery practices such as handling, crowding, hauling, drug treatments, and (particularly during stocking) unfavorable or fluctuating temperatures and water chemistry. This stress is superimposed on that due to the previously mentioned "normal" conditions in the aquatic environment. When recirculation systems are in use, unique conditions of water chemistry can occur that will cause additional debilitation. These include fluctuating exposure to low levels of un-ionized ammonia (NH_3), nitrite (NO_2-), nitrate (NO_3-), carbon dioxide, and gas supersaturation; frequently accompanied by elevated water temperatures (to accelerate growth). Furthermore, pond loadings and population densities are often dictated by economic rather than biological considerations (Piper, 1981).

Water Quality Effects

Because of the many physiological, chemical, and behavioral factors that affect the tolerance of fish to alterations in the aquatic environment, it is difficult to recommend optimum water chemistries, temperatures, and flows for all aquacultural systems. One reason is that the effects of water chemistry vary with the species, size, and age of the fish, and with its previous history of exposure to each variable in question; a second reason, is that the water chemistry itself has a strong influence on the toxicity of any contaminants present. For example, the toxicity of most heavy metals is substantially reduced in waters of total hardness greater than 100 mg/l (as $CaCO_3$). Water temperature and dissolved oxygen can influence toxicities by affecting ventilation rates, and hence the amount of contact of dissovled substance with the gill epithelium. A final reason is that most present water quality information is based on toxicity rather than on fish health considerations. There is, however, limited information available indicating that in soft water recirculation hatcheries, mineral additions to increase the dissolved Cl^- and Ca^{++} levels will improve smolt functionality and general fish health. In spite of above reservations, however, there is general agreement on some environmental chemistry limits. The values given in Table 1 would probably be considered reasonable for salmonid aquaculture in either flow-through or recirculation systems.

Considering anadromous fish as a special case, it is becoming apparent that the environmental conditions required to optimize the parr-smolt transformation are somewhat more exacting than previously believed. Water chemistry, temperature, and photoperiod can all have major effects on smoltification and early marine survival (Wedemeyer, et al., 1980a). One example, particularly pertinent for resource managers, is the effect of exposure to trace heavy metal during rearing (Lorz and McPherson, 1976). The development of the gill ATPase enzyme system of parr- and pre-smolts is apparently inhibited by levels of dissolved heavy metals, singly and in combination, that are well within presently accepted safe limits for

Table 1. Environmental conditions recommended to minimize stress and promote health and quality of salmonid fish in aquacultural systems (Environmental Protection Agency, 1976; Wedemeyer, et al., 1980b).

Water chemistry characteristic	Recommended environmental limits
Acidity	pH 6-9
Alkalinity	at least 20 mg/l (as $CaCO_3$) in flow-through; 100 mg/l in recirculation systems
Ammonia (NH_3)[1]	0.02 mg/l (un-ionized form)
Cadmium[2]	0.4 µg/l in soft water (100 mg/l as $CaCO_3$)
Cadmium[3]	3 µg/l in hard water (100 mg/l as $CaCO_3$)
Chromium	0.03 mg/l
Copper[4]	less than 5 µg/l in soft water; 30 µg/l in hard water
Mercury	0.05 µg/l average, 0.2 µg/l maximum
Nitrite (NO_2-)	100 µg/l, soft water; 200 µg/l, hard water
Nitrate (NO_3-)	10 mg/l, soft water
Pesticides (µg/l)	
Aldrin	0.003
Chlordane	0.010
DDT	0.001
Heptachlor	0.001
Malathion	0.100
Parathion	0.040
Polychlorinated biphenyls	0.002 mg/l (as Aroclor 1254)
Supersaturation[5]	Maximum total gas pressure 110% of air saturation value (sea level)
Temperature	15°C for growth, normal smoltification and migration of anadromous salmonids except steelhead trout, Atlantic salmon. 13°C for spawning, hatching, and egg development of Pacific salmon and steelhead trout; and normal smoltification, growth, and migration of steelhead trout, Atlantic salmon. 9°C for spawning, egg development and hatching of Atlantic salmon.
Total suspended, settleable solids	80 mg/l or less

[1]A maximum of 0.005 mg/l is healthier for salmonids.
[2]To protect salmonid eggs, fry, and normal smoltification.
[3]To protect salmonid eggs and fry.
[4]Copper concentrations exceeding 5-10µg/l may suppress gill ATPase and compromise smoltification of anadromous salmonids.
[5]Not to exceed 102-103% (for eggs to prevent coagulated yolk or white spot disease?.

non-anadromous fish. For example, chronic exposure to copper at only 20 to 30 µg/l during the parr-smolt transformation of coho salmon (*O. kisutch*) partly or completely inactivates gill ATPase function. The biological damage is not apparent until the fish are moved into salt water, at which time substantial mortalities occur. A more subtle but equally devastating consequence is the suppression of normal migratory behavior. Similar results were reported by Davis and Shand (1978) for sockeye salmon (*O. nerka*). Exposing smolts for 144 hours to copper at 30 µg/l impaired hypoosmoregulatory performance (as revealed by the seawater challenge test) and some mortality resulted).

Cadmium levels of more than 4 µg/l in freshwater also result in a dose dependent mortality if exposed coho salmon smolts are transferred directly into 30 parts per thousand (ppt) seawater (Lorz, et al., 1978a). However, if a five-day freshwater recovery period is allowed, saltwater survival returns to normal. In contrast to the effects of copper, sublethal exposure to cadmium or zinc during rearing apparently does not adversely affect migratory behavior. However, if dissolved copper is present as well, synergistic effects occur and both migratory behavior and gill ATPase development are suppressed. Again, few changes in growth or behavior are apparent and the smolts appear to be normal in the hatchery.

Exposure to nickel or chromium at concentrations up to 5 mg/l for 96 hours does not adversely affect migratory behavior or seawater survival. However, sublethal mercury exposure does result in a dose dependent seawater mortality (Lorz, et al., 1978a).

The fact that smolts exposed to heavy metals would be unable to migrate directly into the ocean would tend to increase their residence time in the river and estuary with consequent

increased exposure to predation and to diseases such as vibriosis and viral erythrocytic necrosis.

Less is known about the effects of low level heavy metal exposure on smoltification and migration of sockeye, pink (*O. gorbuscha*), chum (*O. keta*), or fall chinook (*O. tshawytscha*) salmon, all of which have freshwater behavior patterns that are different from these coho. However, Servizi and Martens (1978) did report that mortality, hatching, and growth of sockeye salmon during the egg to fry stage were not affected by continuous exposure to 5.7 μg/l cadmium. Sensitivity increased during development from the alevin to the fry stage. For copper, the incipient lethal level during the egg to fry stage varied between 37 to 78 μg/l for sockeye salmon and 25 to 55 μg/l for pink salmon. Hatching mortality occurred only at concentrations that were also lethal to eggs and alevins. In pink salmon, mortality occurred when copper concentrations in tissues reached 105 mg/kg in eggs or 7 mg/kg in fry. For (inorganic) mercury, exposure to concentrations of only 2.5 μg/l caused malformation of embroyos of sockeye and pink salmon that occurred when tissue concentrations reached 2 mg/kg.

In addition to exposure to trace heavy metals (normally due to drainage from mineral deposits, or to non-point source industrial pollution), increasingly intensive forest and range management, and agricultural practices are resulting in chronic low-level herbicide and nitrate concentrations in waters used by juvenile salmonids. For example, coho salmon smolts exposed for 96 hours to Tordon 101 (a formulation of picloram and the dimethylamine salt of 2,4-D used for brush, weed, and vine control on non-crop lands, including rights of way) at 0.6 to 1.8 mg/l just before they were released did not migrate as successfully as did the control group (Lorz, et al., 1978b). Other 2,4-D and 2,4,-5-T formulations, such as the esters used for control of Eurasian watermilfoil, may also inhibit migratory behavior of smolts. The potential for such deleterious effects should be considered in decisions involving the applications of herbicides.

Ammonia, urea, and other potential sources of nitrate are entering natural waters from domestic or industrial sewage, animal feedlots, seepage, return flows from agricultural lands, and the aerial application of fertilizers to forests and rangelands.

Chronic nitrate exposure at concentrations of more than about 10 mg/l have been shown to be mildly toxic to the developing eggs and early fry of rainbow and steelhead trout (Kinchloe, et al., 1979). Chinook salmon and Lahontan cutthroat trout (*S. clarki*) were adversely affected at 20 mg/l and higher. Coho salmon are sufficiently resistant that nitrate exposure during incubation is probably of no concern, even in recirculation hatcheries using biological filtration.

Temperature, Crowding, Handling, Scale Loss

In recirculation facilities, it is economically feasible to use heated water to accelerate growth and shorten the normal time needed to produce smolts (Saunders, 1976). However, recent research has shown that such artificial temperature regimes must be used with care because they can influence the pattern of smoltification, as well as increase growth. Both hypoosmoregulatory ability and development of gill ATPase function can be affected. In certain species, elevated temperature not only can accelerate the onset of smolting but also can hasten the process of desmoltification, thus shortening the duration of the smolting period (Figure 1). Coho salmon show a delayed rise in gill ATPase function at 6°C, a more nearly normal pattern at 10°C, and a precocious development pattern at 20°C (Zaugg and McLain, 1976). Experience has shown that temperatures up to 15°C are acceptable for the acceleration of smolting so that juvenile coho salmon can be introduced into seawater in their first year; however, as shown in Figure 2, desmoltification is also accelerated, which reduces the period of time during which the fish can be safely released (Novotny, 1975; Donaldson and Brannon, 1976; Clarke and Shelbourn, 1977). Juvenile fall chinook salmon also revert to the parr form more rapidly at elevated water temperatures (Clarke and Blackburn, 1977). Ac-

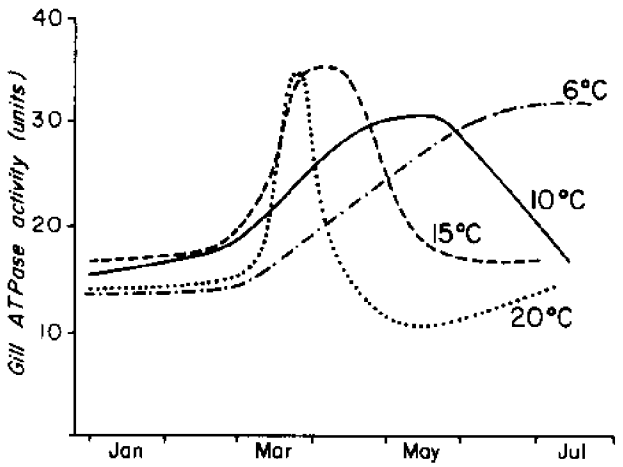

Figure 1. The effect of water temperature on the pattern of gill ATPase activity development in juvenile coho salmon (redrawn from Zaugg and McLain, 1976).

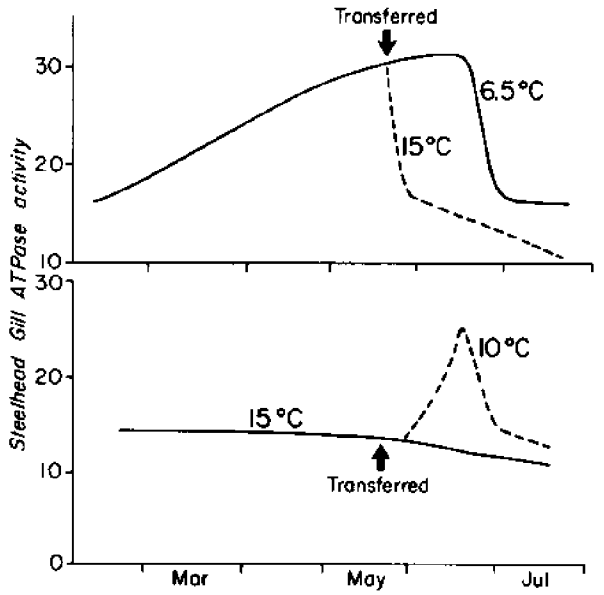

Figure 2. Effect of water temperature on gill ATPase activity in yearling steelhead trout. A. Reared at 6.5°C, then (arrow) transferred to 15°C. B. Reared at 15°C, then (arrow) transferred to 10°C.

celerated desmoltification during rearing at elevated temperatures can be at least partly overcome by holding juveniles in dilute seawater at salinities of 10 to 20 ppt (W. C. Clarke, unpublished data, cited in Wedemeyer, et al., 1980a).

For certain salmonids, acceleration of growth by the manipulation of temperature is less feasible. Anadromous rainbow trout (steelhead) are particularly sensitive to the elevated rearing temperatures sometimes used to accelerate their growth (Figure 2). Smoltification can be inhibited at temperatures of 13°C or higher (Adams, et al., 1973 and 1975; Zaugg and Wagner, 1973). For Atlantic salmon, laboratory experiments have shown apparent

smoltification at temperatures as high at 15°C (Saunders and Henderson, 1970; Komourdjian, et al., 1976). However, native Atlantic salmon stocks show their greatest downstream migrant activity as the temperature warms to 10°C, and runs are normally over before the temperature reaches 15°C. Thus, it is prudent to assume that the temperature responses of Atlantic salmon are similar to those of steelhead trout.

A second recently discovered hatchery stress factor is the effect that pond or raceway loadings can have on smoltification. Normal ATPase development and marine survival of anadromous salmonids can be inhibited if population densities are too high (Strange, et al., 1978; F. Sandercock, Fisheries and Oceans, Canada, personal communication). Extensive experimentation is now in progress in North American to generate the rearing density data needed to balance fish production requirements against adverse effects on smolt functionality. The guidelines summarized in Table 2 are used by the Washington Department of Fisheries, and have given good results under the indicated limitations (J. Wood, personal communication, cited in Wedemeyer, et al., 1980b). In applying these guidelines, recirculating ponds (as would be expected) must be operated at reduced population densities. For example, maximum loadings for (spring) chinook salmon in one type of deep recirculating raceway are about 0.9 kg fish per l/min water inflow with a space density requirement of 0.05 kg fish per m^3 of volume per millimeter fish length. Increasing the loading and space density factors to 1.2 and 0.07 respectively will result in the production of additional "smolts" but will not necessarily give increased adult returns.

Table 2. Recommended maximum loading densities for Pacific salmon, expressed as kilograms of fish per l/min water inflow per millimeter fish length. Information is not available for other temperatures, sizes or species. Loadings must be reduced in recirculating raceways (J. Wood, personal communication, cited in Wedemeyer, et al., 1980b).

Water Temperature (°C)	Fish size (mm)						
	COHO SALMON (mm)						
	36	43	74	94	107	117	140
3	0.06	0.014	0.014	0.015	0.018	0.021	0.022
9	0.01	0.012	0.011	0.014	0.017	0.018	0.017
14	0.008	0.0095	0.008	0.013	0.012	0.013	0.014
17	—	0.006	0.006	0.007	0.0085	0.01	0.009
20	—	—	0.0025	0.0025	0.0035	0.0035	0.0035
	CHINOOK SALMON (mm)						
	38	48	81	104	119	130	155
3	0.01	0.011	0.0095	0.01	0.0115	0.012	0.011
9	0.0085	0.008	0.008	0.008	0.0095	0.01	0.009
14	0.0065	0.006	0.0055	0.0055	0.007	0.0075	0.0075
17	—	0.003	0.0045	0.004	0.004	0.0045	0.0045

The values are final loadings at time of smolt release for fish sizes of 2,200 or fewer per kilogram. Loadings should not exceed 3/4 of the table value before the final pond divisions to ensure that the above loadings will not be exceeded at time of release. In addition, chinook salmon should not be fed in excess of 0.012 kg of food per liter per minute water inflow before they reach a size of 444 per kg; 0.018 kg food/l/min at 222 fish/kg; or 0.024 kg/l/min at larger fish sizes.

The maximum recommended space density for chinook salmon is about 0.18 kg/m^3/mm body length.

The raceway measurements should approximate ratios of 1 unit of depth to 3 units of width to 10 units of length, and the number of water exchanges per hour should be 1.5 to 2.0. Loadings must be reduced in recirculation raceways.

A third hatchery stress factor peculiar to anadromous fish culture is the effect on smolts of handling or hauling. Two types of physiological damage must be considered. First, the stress response itself is more severe in smolts than in parr. Second, various amounts of scale loss commonly occur when smolts are handled, which in turn causes additional physiological

debilitation, especially when they encounter seawater. Bouck and Smith (1979) showed that the loss of 10 percent of the ventral scales of coho salmon smolts resulted in a 50 percent seawater mortality within about ten days. The immediate influx of electrolytes from the seawater (or even salt mixtures being used to mitigate handling stress) results in a life-threatening hyperkalemia and hypermagnesemia in coho salmon smolts (Wedemeyer, et al., 1980a). Even when actual mortalities do not occur, blood electrolyte imbalances resulting from scale loss are debilitating and certainly compromise ability to cope with further environmental stressors.

Disease Treatments

The final stress factors to be discussed in this consideration of current aquaculture practices concern the problem of the side effects of fish disease treatments. Several of the therapeutants presently used to treat anadromous fishes have been shown to concomitantly reduce the ability of smolts to survive in seawater (Bouck and Johnson, 1979). Thus, the treatment can literally be worse than the disease. Ability to hypoosmoregulate is sometimes regained if a freshwater recovery period of about one week can be allowed. Much additional work is needed, but for the present, the data presented in Table 3 can be used as a guideline.

Table 3. Effect of commonly used fish disease therapeutants on ability of coho salmon smolts to survive 10 days in 28 ppt seawater (after Bouck and Johnson, 1979).

	Treatment regimen			Seatwater mortality (%)	
Fish disease therapeutant	Concentration (mg/l)	Time (min)	Treatment period (days)	Direct transfer into seawater	4-day recovery in fresh water after treatment
Copper sulfate	37	20	1	100	20
Formalin	167	60	1	12	0
Hyamine 1622	2	60	4	68	4
Malachite Green	1	60	1	44	12
MS-222	100	6	1	100	12
Nifurpirinol	1.5	60	4	8	0
Oxytetracycline	1	60	1	20	12
Potassium permanganate	2	60	3	80	12

Fish Diseases

The fact that stressful environmental changes can lower the resistance of fish to both infectious and noninfectious diseases has been widely recognized in recent years (Snieszko, 1974; Wedemeyer, et al., 1976). The role of the environment in human and animal diseases has long been understood, and disease prevention is an important part of current public health and veterinary medical programs. Prevention, together with an aggressive immunization program, has been responsible for the virtual elimination of certain diseases in many countries and the eradication of smallpox from the human race.

In a like manner, experience has shown that a wide variety of bacteria, parasites, and other fish pathogens cause mortalities in hatcheries only if unfavorable environmental conditions also exist. These conditions include excessive crowding, handling, dissolved carbon dioxide, nitrite, ammonia, temperature fluctuations, marginal dissolved oxygen, or chronic exposure to trace contaminants in the water (Piper, 1981). Thus, fish diseases usually have more than a single cause and are the end result of the relationship between the pathogen, the fish, and the environment. When this relationship is balanced, good health and growth result. When it is marginal, disease problems and reduced growth will be noted. When it is poor, reduced growth, overt disease, and mortalities occur. Classical examples in aquaculture are fish diseases due to facultative bacterial pathogens such as *Aeromonas*, *Pseudomonas*, and *Myxobacteria* sp., which are continuously present in most hatchery water supplies. Epizootics usually do not occur unless environmental stressors also weaken the defense

systems of the fish. A specific example is bacterial gill disease, outbreaks of which frequently respond to simply reducing the population density in the ponds. Other examples of stress-mediated infectious fish diseases commonly encountered in aquaculture include vibriosis (*Vibrio anguillarum*), the bacterial hemorrhagic septicemias, and parasite infestations such as costiosis (*Costia necatrix*).

The occurrence of chronic fish disease can be used, in turn, as an indicator of the presence of single or multiple stress factors. In freshwater, diseases that usually will not respond to treatment unless existing adverse environmental conditions are also corrected include *Saprolegnia* and other fungus infestations, the previously mentioned low-grade chronic bacterial infections, the hemorrhagic septicemias and gill disease. In marine or estuarine aquacultural systems, viral erythrocytic necrosis and vibriosis are potentially useful indicator diseases. A more complex list, together with a description of the environmental conditions implicated in their occurrence, is presented in Table 4.

Assessing Tolerance Limits of Fishes to Environmental Stressors

It is somewhat difficult to measure unequivocally—short of using death as the end point—the severity of the stress of intensive fish culture, or environmental alterations due to development projects, or the tolerance limits for adaptation. This shortcoming is especially true of methodology for assessing the severity of multiple stress factors. Nevertheless, experience has shown that certain of the blood chemistry, tissue, whole animal, and population changes resulting from the primary, secondary, or tertiary aspects of the stress response can be quantitated and used as indices. As would be expected, some of the physiological tests used to measure these changes require complex laboratory equipment. However, others, such as the occult hemoglobin test for detection of acute stress, require only prepackaged reagents, and thus can be performed in any modestly equipped facility (Smith and Ramos, 1976).

Certain of these physiological tests can also be employed to detect incipient disease, sublethal effects of environmental contaminants, and to monitor nutritional status in fish nutrition research. Examples of tests having such potential are the C-reactive protein determination for the detection of incipient disease, and the ketone-mucus test as an index of nutritional status (Ramos and Smith, 1978a and 1978b). Several of the more practical stress assessment methods will be discussed briefly here. A more complete list is summarized in Table 5, together with interpretive guidelines.

Physiological Testing Methodology

As mentioned, certain aspects of the stress response, such as severity, the time needed for recovery, and tolerance limits for adaptation can be measured by means of existing biomedical test procedures. Examples of blood chemistry tests that are practical include determinations of the stress hormones themselves, or secondary blood chemistry changes such as hyperlacticemia, hypochloremia, or hyperglycemia (Schreck, 1972; Wendt and Saunders, 1973; Hattingh, 1976; Mazeaud, et al., 1977; McLeay, 1977; Wedemeyer and Yasutake, 1977). Hematological tests that are useful as indices include measurements of blood clotting time, and the differential leucocyte count (McLeay, 1975b; Casillas and Smith, 1977). Leucocytosis and eosinophilia may occur under certain conditions, but are of only limited usefulness in assessing the stress response because sensitivity is low and variability high. However, leucopenia is regularly observed and a rapid approximate method, the leucocrit, has been developed for its measurement (McLeay and Gordon, 1977). Changes in erythrocyte counts (as approximated by the hematocrit) or in blood hemoglobin are useful as indicators of hemodilution or hemoconcentration. However, the occurrence of anemias or stress polycythemia may make interpretation difficult (Soivio and Oikari, 1976).

Quantifiable tissue changes that occur as the result of the stress response include depletion

Table 4. Stress-mediated fish diseases common in salmonid aquacultural systems (after Wedemeyer, et al., 1976).

Disease and etiological agent	Environmental stress factors predisposing to disease
Furunculosis (*Aeromonas salmonicida*)	Low oxygen (4 mg/l) crowding; handling in the presence of *A. salmonicida*; handling for up to a month before an expected epizootic.
Bacterial gill disease (*Myxobacteria* sp.)	Crowding; unfavorable environmental conditions such as chronic low oxygen (4 mg/l); elevated unionized ammonia; and particulate matter in water.
Columnaris (*Flexibacter columnaris*)	Crowding or handling during periods of warm (15°C) water if carrier fish are present in the water supply; temperature increase to about 30°C if the pathogen is present, even without crowding or handling.
Kidney disease (*Renibacterium salmoninarum*)	Water hardness less than about 100 mg/l (as $CaCo_3$); diets containing corn gluten or less than about 30% moisture.
Hemorrhagic septicemia (*Aeromonas* and *Pseudomonas* sp.)	Pre-existing parasite infestations such as *Costia*, or *Trichodina*; inadequate cleaning, leading to increased bacterial load in water; particulate matter in water; handling, or crowding; low oxygen; chronic sublethal exposure to heavy metals, pesticides, or polychlorinated biphenyls (PCB's); for carp, handling after overwintering at low temperature.
Vibriosis (*Vibrio anguillarum*)	Handling; dissolved oxygen lower than about 6 mg/l, especially at water temperatures of 10-15°C; brackish water at 10-15 ppt salinity.
Parasite infestations (*Costia*, *Trichodina*)	Overcrowding of fry and fingerlings; low oxygen; excessive size variation among fish in ponds.
Fin and tail rot	Crowding; improper temperatures; nutritional imbalances; chronic sublethal exposure to PCB's or to suspended solids at 200-300 mg/l.
Coagulated yolk (white spot) disease of eggs and fry	Rough handling; malachite green containing more than 0.08% zinc; gas supersaturation of 103% or more; mineral deficiency in incubation water.
Hauling loss (delayed mortality)	Hauling, stocking, handling, in soft water (less than 100 mg/l total hardness); mineral additions not used; CO_2 above 20 mg/l).

Table 5. Outline interpretation of physiological tests to assess the effects of environmental stress on fish health. The examples are based on salmonids but are applicable, with caution, to other fish (Wedemeyer and Yasutake, 1977).

	Clinical test	Possible significance if: Too low	Too high
1.	Ammonia (water un-ionized)	No recognized significance	Gill hyperplasia, predisposition to bacterial gill disease
2.	Blood cell counts		
	a. Erythrocytes	Anemias, hemodilution due to gill damage; impaired osmoregulation	Stress polycythemia, dehydration, hemoconcentration, gill hyperplasia
	b. Leucocytes	Leucopenia due to acute stress	Leucocytosis due to bacterial infection
	c. Thrombocytes	Abnormal blood clotting time	Thrombocytosis due to acute or chronic stress
3.	Chloride (plasma)	Gill chloride cell damage, compromised osmoregulation	Hemoconcentration, compromised osmoregulation
4.	Cholesterol (plasma)	Impaired lipid metabolism	Fish under chronic stress, dietary lipid imbalance
5.	Clotting time (blood)	Fish under acute stress, thrombocytopenia	Sulfonamides or antibiotic disease treatments, affecting the intestinal microflora
6.	Cortisol (plasma)	Interrenal exhaustion from severe stress	Fish under chronic or acute stress
7.	Glucose (plasma)	Inanition	Acute or chronic stress
8.	Glycogen (liver or muscle)	Chronic stress, inanition	Liver damage due to excessive vacuolation; diet too high in carbohydrate
9.	Hematocrit (blood)	Anemias, hemodilution	Hemoconcentration, dehydration; stress polycythemia, gill hyperplasia
10.	Hemoglobin (blood)	Anemias, hemodilution, nutritional disease	Hemoconcentration, dehydration, stress polycythemia, gill hyperplasia
11.	Lactic acid (blood)	No recognized significance	Acute or chronic stress, swimming fatigue
12.	Methemoglobin (blood)	No recognized significance	Excessive NO_2- in water or use of O_2 instead of air in fish hauling trucks
13.	Nitrite (water)	No recognized significance	Methemoglobinemia in fish population
14.	Osmolality	External parasite infestation, exposure to heavy metals, hemodilution	Dehydration, salinity increaes in excess of osmoregulatory capacity, stress-induced diuresis, lactic acidosis
15.	Total protein (plasma)	Infectious disease, kidney damage, nutritional imbalance, inanition	Hemoconcentration, impaired water balance

of muscle and liver glycogen, and interrenal vitamin C. Histologically, the extent of any interrenal hypertrophy may be semiquantitated microscopically by estimating nuclear diameters and cell size (McLeay, 1975a). Several whole-animal responses to stress factors occur that have long been used in hatcheries as indices of overall environmental conditions. These include changes in feeding behavior, decreased growth and food conversion or increased fish disease morbidity and mortality rates.

In recent years, a beginning has been made in methodology for biological monitoring of environmental quality in natural waters. Changes in fish population characteristics can be measured (*a posteriori*) including intrinsic growth rate, disease incidence, spawning success, longevity, egg size, or recruitment to succeeding life stages. Unfortunately, these methods are difficult to use to predict what the environmental impacts will be from proposed develop-

ment projects, especially when multiple stress factors will be involved (Snieszko, 1974; Hodgins, et al., 1977; Ryan and Harvey, 1977; McFarlane and Franzin, 1978).

Monitoring Stress During Rearing: A Case History

Fish reared in recirculation hatcheries using heated effluents are exposed to environmental conditions (water chemistry and temperature complexes) that usually do not occur in conventional systems. Thus, health and quality tend to be more easily compromised. One research objective of the U.S. Fish and Wildlife Service National Fishery Research Center in Seattle is to develop improved methods of biological monitoring to detect the early stages of environmental stress so that preventive measures can be taken. The case history reported here involves a comparative fish health examination of anadromous rainbow trout (steelhead) reared in a recirculation system using biological water reconditioning or in a conventional flow-through system at the same hatchery. Since construction, fish diseases in the re-use system have been chronic, and post-release smolt survival less than desired.

The results are shown in Table 6. Although the recirculation system produced smolts that were physically larger than those in the flow-through system, the tests revealed the presence of several debilitating physiological abnormalities, including inadequate liver glycogen, hypochloremia, hyponatremia and leucopenia, accompanied by moderate dehydration.

Table 6. Fish health in flow-through and recirculation systems at the same hatchery; steelhead trout, 17-19 cm. Water temperature, pH, D.O., nitrite, and ammonia levels were similar in each system. Physiological test values are given as $\bar{x} \pm$ SD; 30-40 fish per group, * indicates abnormal test results (Wedemeyer, 1980).

Parameter	Flow-through system	Recirculation system
Length (cm)	17 ± 2	19 ± 2
Weight (g)	48 ± 13	60 ± 17
Hematocrit (% packed cells)	40 ± 3	45 ± 7
Hemoglobin (g/100 ml)	9 ± 3	9 ± 1
Buffy coat thickness (microns)	138 ± 6	65 ± 6*
Plasma water (% moisture)	94 ± 1	89 ± 3*
Plasma chlorides (meq/l)	127 ± 6	105 ± 28*
Plasma sodium (meq/l)	146 ± 7	130 ± 23*
Plasma potassium (meq/l)	4 ± 1	5 ± 2
Liver glycogen (mg/100 g)	67 ± 41	23 ± 20*
Plasma osmolality (mOsm)	297 ± 12	294 ± 22

Specifically, plasma chloride and sodium levels were both too low and too variable, indicating osmoregulatory dysfunction and acid-base imbalances. These findings are symptomatic of excessive dissolved CO_2 and inadequate dissolved minerals. Water analyses revealed very low chloride levels, together with total CO_2 concentrations of up to 20 mg/l, in the ponds.

The problem is being solved by a combination of water chemistry manipulations to increase dissolved salts, and modified hatchery practices to improve biofilter operation, and reduce ammonia, nitrite, and carbon dioxide.

Physiological disturbances of the magnitude shown in Table 4 are examples of impacts from multiple stressors that are debilitating, but not lethal while the fish remain in the protected hatchery environment. Physiological testing during rearing offers an efficient method of identifying incipient, debilitating, physiological dysfunctions so that corrective action can be taken and fish of optimum health and quality produced at lowest economic cost.

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Increasing Adult Returns of Hatchery-Produced Coho Salmon Through Optimization of Time and Size at Juvenile Release

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Abstract

Coho salmon yearlings were raised in six ponds at Rosewall Creek, Vancouver Island, British Columbia, from which releases were made at four times (14 April, 12 May, 10 June, 8 July 1975). Prior to each release, a portion of the juveniles in each pond were graded into three size groups (small, medium, large) on the basis of size distributions in each pond. These were nose-tagged according to size group, pond, and release date, and marked by adipose fin removal. Fifty-seven groups were released in all. Returns of adults and precocious males (jacks) to the weir and to the fishery (commercial and sport) were subjected to response surface analysis. Predicted maximum adult returns of 43.5 percent (to the weir and fishery) were associated with computed release of 25.1 g coho juveniles on the 173rd (Julian) day from 1 January (22 June 1975). There was a significant interaction between release time and size: maximum returns from early (14 April) releases would be expected from release of 16 to 17 g juveniles. Averaged returns of jacks (ponds 1 to 6) ranged from 0.0 to 4.65 percent). Predicted returns of jacks would be maximized from early release of large juveniles (e.g. approximately 20 g in April). Benefit-costs at the center of the time and size of release surface for maximum adult returns were estimated at 12.2:1. Maximum benefit-costs on the surface were calculated for release of smaller juveniles between 17 and 22 June 1975. Benefit-costs associated with releases on the path of joint optimality between the two centers rose from 12.2:1 (maximum adult returns) to a calculated 16.5:1 for release of 7.5 g juveniles on 17 June 1975. The results are seen as inputs and outputs of a biological system whose components are not yet well understood. Some of the potential unknowns in the system are discussed in relation to possible ways of increasing the efficiency and cost-effectiveness of salmonid hatchery technology.

Introduction

A federal/provincial salmonid enhancement program began in British Columbia in 1977 with the objective of increasing the size of salmonid stocks. Strategies used in the program include enhancement by artificial propogation, by management of the fishery, by modifications to the natural environment, and by habitat protection. The strategy of artificial propagation includes the construction and operation of spawning channels, incubation boxes and hatcheries, particularly for the five species of Pacific salmon (*Oncorhynchus*) found in British Columbia, and the steelhead (*Salmo gairdneri*). One of the research objectives of biologists associated with the program has been to increase our understanding of the biology and culture requirements of the species involved, with a view to optimization of hatchery technology. Included among the numerous questions whose understanding could increase the efficiency of hatchery production is that of the influence of timing and size of juveniles at release on the success of adult returns.

There is evidence that survival of juvenile coho (*O. kisutch*) and chinook (*O. tshawytscha*) salmon is influenced by size of smolts at release (Wallis, 1968; Johnson, 1970; Hopley and Mathews, 1975; Hager and Noble, 1976). Both timing and size of juveniles at release appear

to infuence survival and size of returning adult coho salmon (Hopley and Mathews, 1975). To examine the question further, controlled releases of coho salmon juveniles were made from an experimental facility on Rosewall Creek, Vancouver Island, British Columbia, in 1975. Details of the release and subsequent returns were reported earlier (Bilton, 1978 and 1980). This paper summarizes some of the major findings to be reported in full elsewhere (Bilton, et al., MS).

Materials and Methods

Coho salmon eggs from Big Qualicum River stock were eyed at the Big Qualicum River Hatchery and transferred to the Rosewall Creek experimental facility. The resulting fry were divided among six Burrow's ponds and fed Oregon Moist Pellets (OMP) throughout the rearing period. It was proposed that the fish from each pond be divided into three size categories, marked accordingly, and released at four different times. The size ranges for the three categories (small, medium, large) were determined through preliminary sampling. The six pond populations, three size groups, and the first three releases provided a total of 54 individual groups comprising about 122,000 juveniles. The 54 groups were tagged distinctively with coded wire nose tags, and the tagged fish were marked for identification by removal of the adipose fin. Before each release, a sample of each group was obtained for information on length, weight, sex, and tag retention. About 7,000 fish remained after the third release. These were graded into three size categories as before, tagged and marked to provide the fourth release. Hence, 18 groups were released on each of three dates: 14 April, 12 May, 10 June 1975; the remaining three groups in the fourth release were liberated on 8 July 1975. The resulting 57 release groups totalled about 129,000 tagged fish (Table 1).

Precocious males (jacks) returning to Rosewall Creek were recovered in the fall of 1975, and the adults in the fall of 1976. All marked fish returning to the Rosewall Creek weir were sampled (length, weight, sex, age) and the heads retained for tag recovery.

Tagged fish were recovered from the commercial fishery (net and troll) by random sampling of catches at a target intensity of 20 percent (Heizer, et al., 1978). Estimates of the total number of fish taken in each tagged group by the fishery were obtained from the actual number of tags recovered, the sampling effort, and the marked-to-unmarked ratios in the catches.

Tagged fish returns in the sport fishery were recovered through the Georgia Strait head recovery program (Argue, et al., 1977). An "awareness factor" of 0.28 was used to estimate the total number of Rosewall Creek tags recovered in the sport fishery. The awareness factor was assigned on the basis of an area-to-area comparison with Puget Sound fisheries (Argue, et al., 1977).

The total returns obtained from the 57 release groups, estimated as indicated previously, were subjected to response surface analysis, using as a model the second-order nonlinear polynomial:

$$Y = b_0x_0 + b_1x_1^{\alpha_1} + b_2x_2^{\alpha_2} + b_{11}x_1^{2\alpha_1} + b_{22}x_2^{2\alpha_2} + b_{12}x_1^{\alpha_1}x_2^{\alpha_2}$$

where x_1 = time of release in days from 1 January (Julian days)
x_2 = mean weight of juveniles in the release group (grams)

The procedure provides an effective way of determining the shape of the response surface in the region of the two-dimensional (x_1, x_2) factor space from which the sample points are taken (Lindsey, et al., 1970). Maximum likelihood estimates of the coefficients and power parameters of the polynomial were obtained and contours of the resulting surfaces were generated in units of the response Y.

Table 1. Mean weight (g) and number of juveniles in groups of fish graded into three size groups (small, medium, large), tagged differentially and liberated from six ponds at four different times.[1] Small, medium, large fish: respectively the lower 25, middle 50 and upper 25 percent of size range in each pond found by sampling prior to tagging. Release dates show Julian days (days from 1 January 1975).

Release date (Julian days)	Size group	Pond 1	2	3	4	5	6
14 April (104)	S	5.1	9.0	9.2	7.0	9.6	10.6
		1243	1627	1788	1779	1404	1776
	M	8.1	11.6	12.9	9.6	12.6	14.2
		3740	3867	3594	3614	3585	3596
	L	10.9	15.4	16.1	13.3	16.7	18.8
		1771	1657	1787	1785	1762	1778
12 May (132)	S	8.3	12.5	11.7	7.7	13.8	14.0
		1873	1871	1873	1868	1865	1830
	M	11.9	15.7	15.1	11.0	18.1	18.5
		3639	3701	3735	3784	3681	3673
	L	15.1	20.8	19.2	15.3	23.9	25.8
		1881	1909	1859	1881	1859	1839
10 June (161)	S	12.3	15.6	14.4	12.3	17.3	17.6
		1492	1642	1358	878	1484	1221
	M	16.0	20.1	18.9	16.3	22.1	23.6
		2586	3937	3359	1853	3451	2220
	L	20.2	28.3	25.1	19.9	28.7	31.8
		1367	1896	1898	1486	1898	1172
8 July (189)	S	14.9					
		1432					
	M	24.8					
		3704					
	L	33.1					
		1801					

[1]Fish remaining in the six ponds after the 10 June release were pooled and subsequently graded, tagged, and released on 8 July.

A number of such response functions were generated from the data (Bilton, et al., MS); three of those are presented here:

Y_1 = percent return of adult coho (excluding jacks).
Y_2 = percent return of jacks only.
Y_7 = benefit-costs associated with returns (percent) of adult coho.

The response Y_7 requires further explanation. Cost of production of the juveniles in each of the four releases was calculated earlier by Bilton and Jenkinson (1976). Returns were assigned one value (Can. $0.90/lb), that at the Rosewall Creek weir. The costs, as derived, are a simplified measure of investment. The benefits, also, no doubt are conservative. Nevertheless, the benefit-cost ratios obtained provide a valid means of comparing outcomes over the factor space considered. For production hatcheries, more complex cost and benefit functions undoubtedly would be used to generate the ratios.

Finally, the surfaces generated for Y_1 and Y_7 are compared. It will be seen that the two surfaces have maxima at different locations in the release time/release weight factor space (Figure 3). By solving a joint optimality problem for the Y_1 and Y_7 response functions, a path may be described between the centers of the two surfaces. The points from which the path is constructed represent loci in the release time/release weight factor space at which tangents may be drawn jointly to touching pairs of Y_1, Y_7 contours. The resulting "path of joint op-

timality" provides estimates of a) benefit-costs associated with maximum predicted adult returns, b) adult returns expected in association with maximum predicted benefit-costs, c) intermediate percent return, benefit-cost estimates between the two surface centers, and d) release times and release weights associated with each selected Y_1, Y_7 joint optimal response estimate on the path.

Results

Returns of adult and jack coho and associated benefit-cost ratios for the 57 release groups (Table 1) are summarized in Table 2. For convenience of presentation, the mean weights of the juveniles from the six ponds contributing to the first three releases (14 April, 12 May, 10 June) have been averaged to provide data on 12 releases—three size groups (small, medium, large) liberated on each of the four release dates. In general, the data show that highest percent returns of adults, and of total returns, occurred for the 10 June release. Benefit-cost ratios also are highest for the June release. In addition, there is strong evidence that returns of jack coho are lowest in the "small" and highest in the "large" juvenile groups. The higher return of jacks associated with release of "large" juveniles tends to reduce their corresponding benefit-cost ratios. This is demonstrated, for example, in a comparison of the returns and benefit-cost ratios for the "medium" and "large" juvenile releases of 10 June. The influence of time of release on subsequent returns, where juvenile size at release is fairly constant, may be judged by comparing returns for the groups averaging about 15 g at release (large, 14 April; medium, 12 May; small, 10 June and 8 July). For these groups, return of adults rises to a maximum in the June release, then declines; the proportion of jacks returning from the same groups rises to a maximum in the May release, then declines.

Table 2. Estimated total returns to the weir and fishery (%) of adult and jack coho, and associated benefit-cost ratios for the release groups of Table 1. For convenience only the results are shown as averages over the six ponds for the release groups of small, medium and large fish at the four times of release; computations, however, are based on returns to all (57) groups.

Release date	Julian days	Group	Average release weight (g)	Returns (%)			Benefit-cost ratios		
				Adults	Jacks	Total	Adults	Jacks	Total
	104	S	8.4	8.39	0.09	8.48	5.01	0.01	5.02
14 April	104	M	11.5	7.55	0.16	7.71	3.38	0.01	3.39
	104	L	15.2	5.82	0.50	6.32	2.10	0.03	2.13
	132	S	11.3	15.51	0.40	15.91	8.76	0.04	8.80
12 May	132	M	15.0	16.60	1.34	17.94	7.38	0.11	7.49
	132	L	20.0	14.75	3.18	17.93	5.16	0.20	5.36
	161	S	14.9	31.85	0.12	31.97	14.22	0.01	14.23
10 June	161	M	19.5	39.94	0.89	40.83	13.95	0.04	13.99
	161	L	25.7	35.46	4.65	40.11	9.77	0.19	9.96
	189	S	14.9	4.82	0.00	4.82	2.17	0.00	2.17
8 July	189	M	24.8	20.17	0.97	21.14	5.44	0.04	5.48
	189	L	33.1	13.71	3.05	16.76	2.83	0.09	2.92

Based on maximum likelihood estimates of the coefficients and parameters of the second-order nonlinear polynomial used as a model, response surface analysis of the data for all 57 releases generates the surfaces shown in Figures 1 and 2. These surfaces show regression contours for predicted returns of adults (Figure 1) and jacks (Figure 2) in relation to time of release and mean juvenile weight at release.

For adult returns (Figure 1), the predicted maximum at the center of the surface (Ys) is 43.5 percent, corresponding to release on day 173.1 (22 June 1975) at a mean juvenile weight of 25.1 g. For the adults the standard deviation of Y over the surface is ± 2.98 percent.

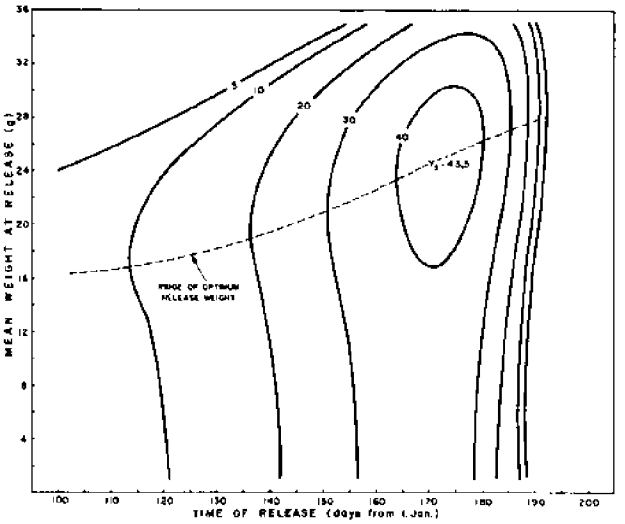

Figure 1 Influence of time of release (Julian days) and mean size at release (g) on total returns of adult coho salmon (to the weir and to the fishery). Contours are shown for 5, 10, 20, 30 and 40 percent return. Predicted maximum return at the center of the surface (Ys) is 43.5 percent, for a release on day 173.2 (22 June 1975) at a mean juvenile release weight of 25.1 g.

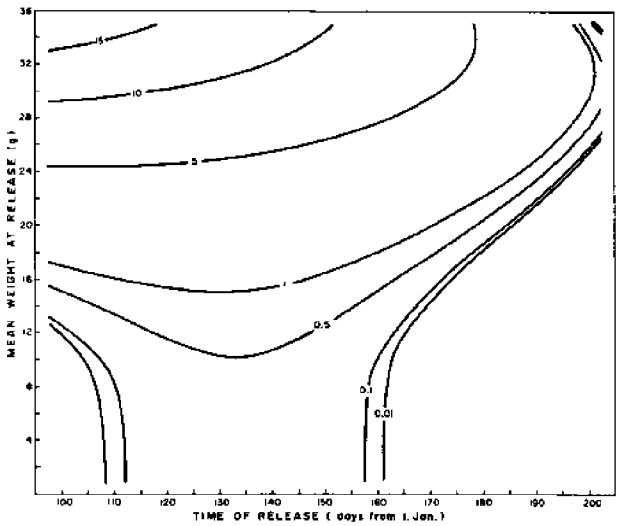

Figure 2 Influence of time of release (Julian days) and mean size at release (g) on total returns of jack coho salmon. Contours (% return) increase toward the upper left corner of the figure. The provisional surface indicates that increased returns of jacks can be expected from early release of larger juveniles.

Hence,the contours are known with a precision of about ± 6 percent (P = 0.05). The surface also shows an interaction between time of release and juvenile size with respect to adult returns. That is, maximum returns occur with early release of smaller juveniles and later release of larger juveniles. This can be illustrated as follows (Figure 1). A curve drawn to connect the points at which tangents to the surface contours are parallel to the axis of mean weight ($\partial Y/\partial x_2 = 0$) provides estimates of release weights for which percent return of adults is maximized at given release times. For the four Julian days of Table 1 (days 104, 132, 161, 189) these weights are, respectively, 16 to 17, 18 to 19, 22 to 23 and 27 to 28 g. The surface also indicates that returns from releases made after approximately day 180 (29 June 1975) can be expected to drop sharply whatever the mean size at release may be.

For returns of jacks (Figure 2), the predicted maximum at the center of the surface is remote from the factor space examined. The standard deviation of Y (percent return of jacks) over the surface is ± 0.59 percent. Hence, the contours of the surface are known with a precision of about 1.2 percent (P = 0.05). For both surfaces (Figures 1 and 2) the estimates of precision refer, of course, to the factor space (time of release, weight at release) encompassing the 57 data points involved. Most significant in Figure 2 is the indication that greater returns of jacks may be expected from early releases of larger juveniles, an outcome that most salmonid culturists would wish to avoid.

Figure 3 represents an overlay of three responses evaluated from the data. A central contour of the surface for percent return of adults (40 percent, Figure 1) is shown (upper right in figure). Several contours are also shown from Figure 2 for percent return of jacks (0.01, 0.5, 5 percent). Also indicated are several central contours of the benefit-cost surface for adult returns (lower right in figure; contours 14, 16; Bilton, et al., MS). Also shown is the path of joint optimality running from the center of the surface for adult returns toward the center of the surface for benefit-costs of adult returns. The center of the latter surface is outside the

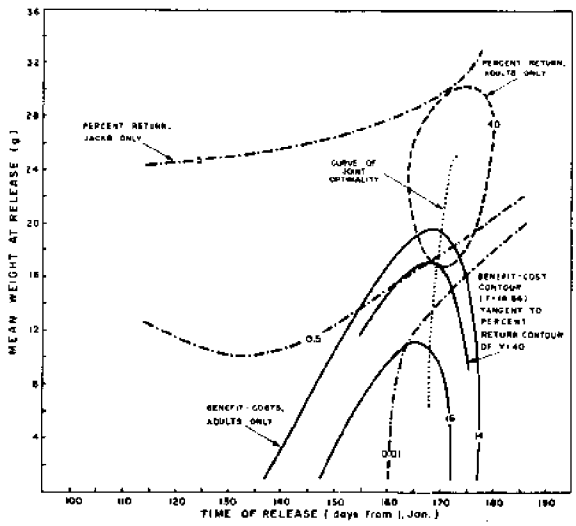

Figure 3 Overlay of computed response surfaces. Shown are 1) a central contour (40%) around the center (Ys) of the surface for percent return of adult coho, 2) several contours (0.01, 0.5, 5%) of the surface for percent return of jack coho, and 3) central contours (14, 16) of the benefit-cost surface for adult returns. The coordinates of points on each surface are those of time (Julian days) and size (g) of juveniles at release. Also shown is a "path of joint optimality," running from the center of the surface for adult returns toward that for benefit-costs of adult returns. Points on this path are optimized jointly between the two centers in terms of time and size at release (see Table 3).

release time/release weight factor space considered, and benefit-cost estimates at low release weights (e.g., less than 6 g) presumably have doubtful value. Nevertheless, the path of joint optimality shows that maximum percent return of adults is achieved at less than maximum benefit-costs. Conversely, maximum or near-maximum benefit-costs are achieved at less than maximum return of adults. In general, moving upward on the path of joint optimality, adult and jack returns increase and benefit-cost ratios diminish. These relations are shown in Table 3, together with associated coordinates of release time and mean weight at release. In general, the following observations are made from the table:

1) At the center of the surface for adult returns a benefit-cost rate of 12.2:1 is predicted.
2) The optimum time of release is relatively constant (about day 170 on the path of joint optimality.
3) Release of smaller juveniles on or near day 170 should result in maximized but lower adult yields, and with increased monetary benefits relative to production costs.
4) Conversely, higher adult returns are coupled with increased returns of jacks, presumably the main reason for the concomitant reduction in benefit-costs.

Table 3. Loci on the path of joint optimality between the center of the surface for percent return of adult coho, and that for benefit-costs associated with adult returns. Each locus is defined in terms of a time of release and mean weight at release at which the adult returns and benefit-costs are jointly determined.

Coordinates of surface		Response	
Release time (days)	Mean weight (g)	Adult returns (%)	Benefit-costs ($/$)
173.1[1]	25.1[1]	43.5	12.2
172.9	24.7	43.5	12.4
172.3	24.0	43.4	12.6
171.7	23.0	43.1	13.0
171.0	21.1	42.4	13.5
169.6	16.9	40.0	14.6
169.1	15.2	39.0	15.1
168.7	13.3	38.0	15.5
168.3	11.0	37.0	16.0
168.0	7.5	36.0	16.5

[1]Locus of center of surface for percent adult returns.

Discussion

The current study raises a number of questions regarding the general applicability of the results to current salmonid hatchery technology. This study identified inputs and outputs of a system whose central biological components are as yet not well understood. Patterns of release and return, and variability within such patterns, are assumed to arise from at least three sources. These are site-specific factors unique to particular hatchery locations, factors whose influence on multiple sites may vary from year to year (annual variability), and basic differences in release and return patterns that are specific to salmonid species. These will be discussed in turn.

First, however, a comment is required on the technique of grading of pond populations into three size groups (small, medium, large) prior to release, and the possible influence this might have on resulting returns of jacks and adults. The technique, as used, may be contrasted with an alternative whereby three individual pond populations are grown at different rates, through temperature control, to achieve three different mean sizes at time of release. In defense of the grading technique used, it happens that many salmonid hatcheries do not have facilities for controlling temperature. Yet, grading must take into consideration the evidence that growth rate and age of return may be influenced by genetic factors. Grading,

then, might accentuate differences in proportions of jacks returning from release groups of small, medium and large smolts, differences that would remain undetected if such groups remained merged in one release population. The question then remains: Would the jack returns from ungraded populations conform with those obtained in this study where grading was used? Inspection of the original data (Bilton, 1978) indicates the proportions of jacks returning from the graded release groups in the April, May, and June releases were not demonstrably different, for a given size, than the proportion returning to a combined population of small, medium, and large release groups having the same mean size. There was one exception among the groups compared: a higher proportion of smolts than expected returned as jacks from the June release of large fish. This can be stated more formally in the language of analysis of variance. For each smolt release, there are two pieces of size information, the mean weight in grams and the group (small, medium, large) from which the smolts came. With the exception of the June release just noted, analysis of variance shows that the second piece of information (group) does not add significantly to the first (mean weight) in explaining jack returns.

A similar analysis of variance shows that the extra information on smolt grouping has no significant influence in explaining any of the adult returns. This result is corroborated by data from independent releases of coho juveniles from four British Columbia hatcheries where grading did not occur (Figure 4). Although site-specific and annual variability likely are imbedded in the hatchery data, the fit of the surface for adult returns (Figure 1) to the hatchery returns appears quite reasonable. In retrospect the data are interpreted to mean that no evidence has been found to support the possibility that genetic factors influenced jack returns through use of the grading technique. Yet, accumulating evidence (Ricker, 1972) suggests hereditary factors are at least partly involved in rate of growth and age of return.

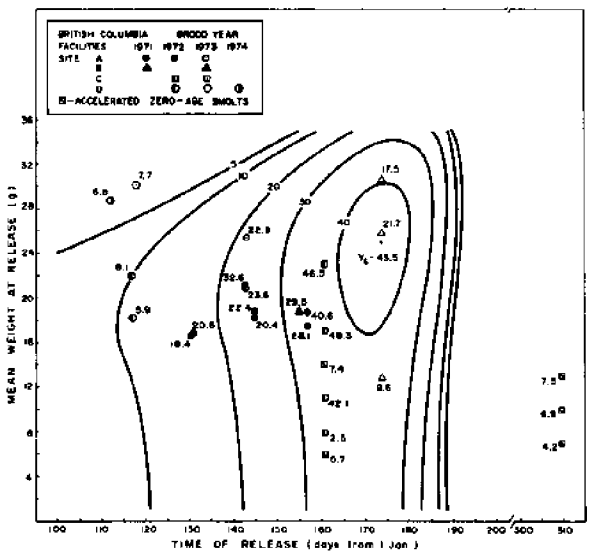

Figure 4 Available data for independent releases of coho juveniles from four British Columbia hatcheries (sites), in four brood years (1972 to 1974), overlaid on the response surface for adult returns generated from the data for the 1975 Rosewall Cr. releases. Ys—center of the contours for the Rosewall data. Numbers beside each hatchery release—percentage of returning adults (fishery and escapement). (Six releases of zero-age smolts, subject to temperature manipulation, rapid growth, and release in the year prior to normal release, also are shown; these are judged not to be comparable with the remaining points on the surface.) (From Bilton, et al., MS).

Hence, the inference that genetic factors could influence jack returns through the grading technique would appear to remain valid. If such were the case, we assume grading could alter the curvature of a response surface for jack or adult returns, but not the general configuration of the surface itself. Given these uncertainties we conclude that different size groups should be obtained, where possible, through temperature control rather than grading. With these comments on potential experimental biases, we may return to the initial discussion of patterns of release and return.

Site-specific factors, such as the annual temperature regime of the water supply, can be fairly constant for a given hatchery site. However, such factors vary considerably from site to site depending on watershed characteristics and geographic location. Hence, optimizing returns to a given hatchery facility in terms of time and size of juveniles at release may require that each facility be "tuned" in relation to its own site-specific characteristics. Conversely, suboptimal characteristics of a water supply and river system on which a hatchery is located may negate the possibility of tuning a facility to maximum efficiency. If the natural water temperature regime is too low it may be impossible economically to achieve the juvenile size required to optimize release and adult return. If temperatures are too high growth may be satisfactory, but early release may be required to avoid seasonal losses from disease associated with higher temperatures.

Second, we suspect that annual variability associated with release and return may be influenced substantially by predation, and by competition for food among the juveniles after liberation. It is speculated that time and size at release may have a major bearing on conditions the juveniles experience in the several months following their migration to and through an estuary and to salt water. The hypothesis is advanced that optimum conditions of time and size at release may be those providing entry of juveniles into the near-shore and coastal ecosystems at a time and size when maximum advantage can be taken of the available food supply in terms of growth rate and survival. The attainment of that advantage may depend on injection of the juveniles into the ecosystem at a time when the presence and density of food organisms of particular types and sizes, and the prevailing temperatures, allow for maximum food intake and growth in relation to the cost of energy expended in foraging. Conditions in turn that maximize food supply, such as may be associated with the spring plankton pulse, may vary geographically and with time in relation to meteorological and oceanographic events affecting the near-shore and coastal marine environments. Variations in these events from year to year and region to region could influence the food supply, feeding opportunities, and hence the growth rate and survival of the juveniles. We infer that conditions providing rapid growth in this period are advantageous. Susceptibility of juveniles to size-dependent predation could be foreshortened by more rapid growth through the "stanzas" of higher vulnerability to predation assumed to be associated with smaller size.

Assuming the hypothesis stated is reasonable, examination of meteorological and oceanographic events, in association with structured releases of juveniles over a period of years, could provide insights into potential correlations between success of adult returns and ecosystem parameters in the initial juvenile marine phase. From such associations simple "flags" might be recognized, such as threshold temperatures in the near-shore environment, signalling most favorable options regarding time of release. The hypothesis, of course, is related to the concept of the "plankton watch" of recent years. A knowledge and use of such correlative factors could serve to reduce annual variations in success of adult returns to hatcheries if indeed the initial juvenile marine phase is a critical one. In addition, the "tuning" of hatcheries by controlling juvenile growth to achieve optimum time- and size-at-release "windows" also could serve to minimize the influence of site-specific variations on success of adult returns.

Third, among the factors mentioned that could influence the success of hatchery returns is the possibility that optimal conditions for time and size at release may vary between species.

That is, each species may have its own optimal pattern of time and size at release. We suspect that the pattern for coho salmon described in this paper will be found to be quite different from that for chinook salmon. The freshwater and estuarine residence of chinook salmon migrants is known to be highly variable. We suspect that optimization of chinook returns to hatcheries through manipulation of time and size at release may await a better understanding of this phase of the chinook life cycle before meaningful advances are made in the success of adult returns. For the steelhead, and on the basis of available evidence, one would expect maximum returns from the liberation of large juveniles. This might not be entirely true if the control variables of release time and size are found to interact, as they so often do in biological systems. In other words, the size of steelhead juveniles providing maximum returns may vary according to the times when releases are made. For chum (*O. keta*) and pink (*O. gorbuscha*) salmon, which migrate to sea as fry, the assumed influence of time and size at release on adult returns is less clear. Japanese experience indicates that greater returns result from liberation of larger fry. Yet the length of time fry may be held in fresh water prior to migration may be limited by eventual changes in their physiological state. Perhaps the influence of time and size at release on adult returns of chum and pink salmon will find its greatest rewards through salt water culture of fry in conjunction with hatchery operations for these species.

To recapitulate, an attempt has been made in this study to assess the relative benefits and costs of hatchery production of coho salmon. Methods of estimating benefit-cost ratios were simplified in the knowledge that their validity was a function of the characteristics of the experimental system to which they were applied. In addition, comparisons of benefit-costs, as defined, are considered valid over the time- and size-of-release factor space for coho in the experiment in question. The results indicate how a hatchery facility may be "tuned" with respect to time and size at release to maximize adult returns, the benefit-costs of adult returns, or intermediate levels of operation between those two optima.

Sources of variability were suggested that could alter the results obtainable. These include site-specific characteristics of hatcheries, and annual variability in adult returns likely attributable to conditions of food supply and predation in near-shore and coastal ecosystems into which the juveniles are injected. Finally, the various species of Pacific salmon, and steelhead, may describe entirely different patterns of adult return in relation to time and size of juvenile release. All of these factors require further examination.

By taking time and size at juvenile release into consideration, new production strategies may suggest themselves for culture of salmonid species at various sites. These strategies will depend on the values for adult return and benefit-costs at the response centers (e.g., Figure 3) AND the extent to which the coordinates of the returns and benefit-cost centers are separated in terms of release time and size. In addition, the use of multiple factor designs and appropriate analytical methods is recommended if similar studies are contemplated at other sites and with other species. Simultaneous variation of time and size at release in this study allowed the information gained to be obtained within one generation of coho salmon. We estimate that similar data obtained by less rigorous methods would require not less than five generations. We suggest that further studies of the influence of time and size of juveniles at release should lead to new information on the biology of Pacific salmon while, at the same time, providing a means for improving the efficiency and cost-effectiveness of current salmonid hatchery technology.

Acknowledgments

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Section V
Genetic Problems in Salmon Enhancement

Proc. No. Pac. Aquaculture Symp.,
Aug. 1980, Anchorage, Alaska

Genetic Potential for Fresh- and Seawater Growth of Net-Pen Cultured Coho Salmon

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Introduction

When compared with domestic livestock breeding programs, our level of expertise in the quantitative genetics of the salmonids in general is probably equivalent to the commercial poultry industry 30 to 35 years ago. This is unfortunate as selection, based on theoretical and practical advances in quantitative genetics, may well serve to enhance future salmonid production (Gjedrem, 1976). Breeding attempts, as historically practiced, with principal reliance on mass or individual selection without an understanding of the genetics of the selected traits may prove to have serious and undesirable consequences (Newkirk, 1978). This problem may become critical with closed broodstocks.

Further, it is inevitable that as salmonid programs become more sophisticated, greater reliance will be placed on developing stocks specifically bred for the particular requirements of each industry. The marine net-pen culture of coho salmon serves as an example. Instead of the normal foraging in ocean pastures, net-pen reared coho must survive and grow rapidly in an environment of high density, artificial diets, and increased handling stress. These fish are being subjected to the initial phases of the domestication process, and the changes induced by captivity will be lost unless progeny of pen-reared individuals are continually exposed to the artificial and natural selective pressures inherent in the new system of rearing. Thus, continued use of seed stock from outside sources cannot be expected to improve the response of the fish to these circumstances. Additionally, the source of seed stock that is traditionally used (state-operated hatcheries) cannot guarantee a consistent and adequate supply for the future because of the expansion of other salmonid programs.

DomSea Farms, Inc., a commercial net-pen operation in Puget Sound, Washington, has anticipated those needs by establishing a coho salmon broodstock to fulfill production requirements. University of Washington personnel with the support of the Washington State Sea Grant Program have collaborated with the firm in developing a program of systematic breeding and selection as well as determining the requirements of captive coho salmon broodstock.

In this report, we have summarized a few of our genetic results from the initial three years of the program. These include genetic and phenotypic variances and correlations, and heritabilities of smoltification and fresh- and seawater growth, determined from analysis of the progeny of two brood years. We will also discuss potential and actual selection gains resulting from the program.

Materials and Methods

Mating Design

Estimates of genetic parameters were derived from measurements on progeny resulting from a balanced, hierarchical design involving the mating of one male with two females (Figure 1). This design was used for two brood years (1977 and 1978). The crosses yielded 70 full-sib and 35 paternal half-sib families in 1977, and 66 full-sib and 33 paternal half-sib families in 1978. In both years, adult fish were held and spawned at a seawater site. Water-hardened eggs were subsequently transferred to incubation and freshwater rearing facilities at Gorst, Washington.

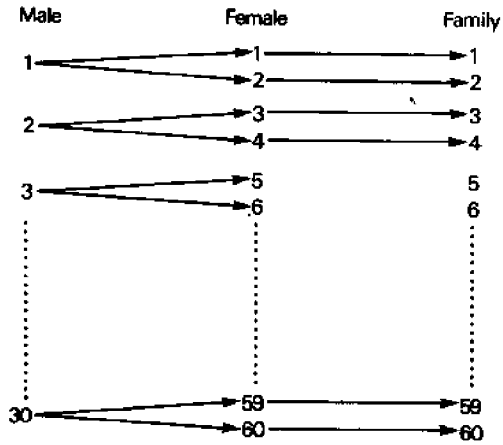

Figure 1. The nested mating design employed to produce families of coho salmon for estimating genetic parameters.

Incubation and Rearing

Individual full-sib lots were incubated in Heath-Techna trays. At yolk-absorption, the 40 families with the largest number of survivors were identified and retained, and the number in each of these families reduced to 500 individuals. Fry from the 40 families were then transferred to 100 liter rearing containers, each family being placed in a separate container.

At approximately 2 g mean body weight, one full-sib family from each paternal half-sib group was marked by excision of a fin (adipose fin for BY 1977 and pelvic fin for BY 1978), and both families were then combined in 1.2 meter diameter fiberglass tanks. Rearing temperatures were maintained at between 13 and 15°C, during the entire freshwater rearing period to promote 0-age or underyearling smoltification (Donaldson and Brannon, 1976). Oregon Moist Pellet and a modified Abernathy diet were fed in sizes and ration levels based on fish size.

Seawater Rearing

Prior to the anticipated completion of freshwater rearing, each full-sib family received a distinctive mark applied by freeze-branding with liquid nitrogen (Mighell, 1969). Fish were then spray vaccinated with a bivalent vibrio vaccine (Schiewe and Hodgins, 1977). After allowance for wound healing and antibody titer elevation, all fish larger than 10 g body weight were transferred to the seawater site for grow-out. During the seawater phase, the fish were reared in floating net pens (20' wide x 50' long x 12' to 15' deep) at a density of approximately 1/2 lb/ft.[3] A significant proportion of the individuals matured at two years of age (24 months post-fertilization). Those individuals selected for broodstock were crossed under a circular mating design (Figure 2).

Data Collection and Analysis

Individual progeny lengths and weights were collected at four, five, seven, 11, and 14 months post-fertilization. Additionally, smoltification as determined by morphological changes and early seawater survival was assessed. Incubation and adult performance were also monitored but will not be discussed in this report.

Freshwater data (75 individuals per full-sib family per measurement period) were analyzed with a balanced, nested analysis of variance (ANOVA) to estimate variance components. In seawater it was not possible to obtain equal numbers per family, so an unbalanced, nested ANOVA was used. The variance components were used to estimate heritabilities (h^2). Analysis of covariance for balanced and unbalanced designs was used to determine phenotypic and genetic correlations. Variances, covariances, and economic values for specific traits were later inserted into a selection index to guide the selection of broodstock.

Selection Program

Adequate freshwater rearing space was available for only 40 full-sib groups of limited size. This necessitated an early culling of families based on incubation performance and of individuals within families on a random basis. At the termination of freshwater rearing, independent culling was again practiced with only those individuals heavier than 10 g being sent to seawater. At approximately four months, post seawater entry, obvious non-smolts (generally fish less than 30 g body weight) were again culled from the population. A selection index based on variances, covariances, and economic weights for freshwater and seawater growth was subsequently used to designate the ten best families. At maturity, ripe individuals within the ten best families were selected as spawners on an individual merit basis. Crosses were then made in accordance with the circular mating design (Figure 2), i.e., males and females from one full-sib group were never mated together.

Results and Discussion

Heritability Estimates

Heritability estimates were calculated for weight and length at three freshwater and two saltwater periods. Data were also taken for the morphological smoltification stage of individuals at pre- and post-seawater entry (seven and 11 months post-fertilization) and heritabilities for those traits were calculated. The heritability estimates for these traits based on the sire component are presented in Table 1. Standard errors, and hence confidence intervals, for all estimates were large because of the limited number of males and females in each brood year. The estimates, however, were fairly consistent for the different measurements between the two brood years, and roughly comparable with other estimates from the literature (Aulstad, et al., 1972; Gall and Gross, 1978; Refstie and Steine, 1978). If regarded as point estimates only, the h^2 values indicate that substantial additive genetic variance is present for all traits concerned and consequently, successful selection is strongly suggested.

Genetic and Phenotypic Correlations

As shown in Tables 2 and 3, standard errors are also high for the genetic and phenotypic correlations and lead to some difficulty in the interpretation of results. There are, however, significant correlations for length and weight at specific periods for both brood years indicating either pleiotropic gene action or close linkage. For example, the genetic correlations (r_G) between pre-seawater entry (seven month) length, weight, and smolt assessment were highly significant for both brood years. This indicates that selection for any of the three traits alone would have some concomitant and positive results on the unselected traits. There is some discrepancy between the different brood years on the effect of selecting for traits be-

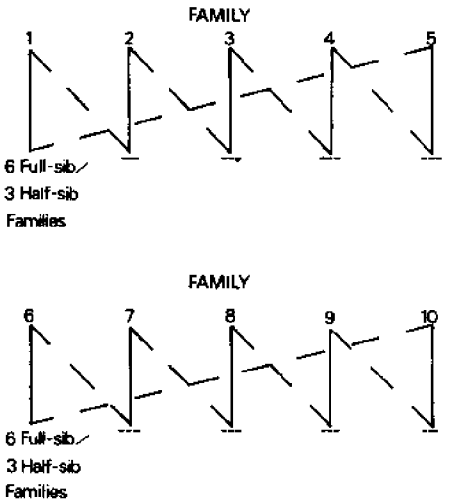

Figure 2. Circular mating design used for individuals from selected families (—— = females —— —— = males).

Table 1. Heritability estimates (based on sire component) on freshwater and saltwater growth measurements for progeny from adults spawned in 1977 and 1978.

Meas. Date	Trait	BY 1977	BY 1978
Freshwater			
Apr. (4)[a]	Weight	.614 ± .314[b]	.351 ± .231
May (5)	Weight	.377 ± .251	.465 ± .217
	Length	.300 ± .237	.564 ± .245
July (7)	Weight	.250 ± .216	.262 ± .202
	Length	.224 ± .211	.370 ± .214
	Stage[c]	.251 ± .165	
Saltwater			
Nov. (11)	Weight	.117 ± .112	.305 ± .180
	Length	.170 ± .128	.377 ± .199
	Stage [c]	.252 ± .141	
Mar. (15)	Weight	.193 ± .111	
	Length	.179 ± .109	

[a]Months post-fertilization.
[b]Standard error.
[c]Based on morphological smolt index (0 = Parr, 1 = Smolt).

tween two measurement periods. According to our results, the genetic correlation between four month weight and seven month smolts was low for BY 1977 (r_G = .1439) but relatively high for BY 1978 (r_G = .6687). Similar differences can be found for other measurements. We suggest that either sampling errors were responsible for the results or, more likely, that this is a reflection of the different genetic constitutions of the two broodstocks.

With the estimates for genetic correlations and heritabilities, it is possible to determine the efficiency of indirect selection for any two traits using the formula:

Relative efficiency = $r_G \quad h_x / h_y$
where r_G is the genetic correlation
between traits X and Y
h_x and h_y are $\sqrt{\ }\ h^2$ for traits X
and Y respectively (Turner and Young, 1969)

Table 2. Genetic and phenotypic correlations among fresh- and seawater traits exhibited in progeny of the 1977 brood. One standard error is given in the parenthesis below each value.[a]

	4W	5W	5L	7W	7L	7S	11W	11L	14W	14L
4W		.7999	.9505	.6167	.7374	.1439	-.0575	-.0715	.3927	.5020
		(.2051)	(.1567)	(.5550)	(.6425)	(.5164)	(.5408)	(.5004)	(.3294)	(.2973)
5W	.4884		.9529	.2488	.4021	-.0280	-.5234	-.5309	.1371	.2575
	(.0351)		(.0432)	(.5563)	(.5629)	(.5362)	(.5890)	(.5411)	(.4001)	(.3784)
5L	.5220	.9553		.0024	.1470	-.2903	-.6573	-.6333	.1948	.3017
	(.0351)	(.0039)		(.6980)	(.7331)	(.6310)	(.6615)	(.6013)	(.4130)	(.3884)
7W	.2902	.1908	.1105		1.0286	.9053	-.0080	-.0559	.5492	.5755
	(.0485)	(.0423)	(.0415)		(.0620)	(.1139)	(.7900)	(.7534)	(.5017)	(.4649)
7L	.3278	.2441	.1615	.9792		.8655	-.1997	-.2230	.5237	.5775
	(.0543)	(.0413)	(.0405)	(.0015)		(.1682)	(1.0596)	(.9908)	(.6435)	(.5996)
7S	.0809	.0661	.0514	.8677	.8801		.1833	.1469	.3932	.4625
	(.0300)	(.0407)	(.0402)	(.0083)	(.0079)		(.5732)	(.5459)	(.4109)	(.3728)
11W	-.0061	.1111	-.1202	.1192	.0857	.1615		1.0157	1.8962	1.6468
	(.0283)	(.0382)	(.0377)	(.0361)	(.0354)	(.0353)		(.0197)	(6.2743)	(5.3034)
11L	-.0130	-.1229	-.1389	-.1046	.0727	.1580	.9621		1.3685	1.0893
	(.0291)	(.0398)	(.0392)	(.0374)	(.0366)	(.0364)	(.0021)		(3.5031)	(2.6183)
14W	.1724	.0681	.0716	.1777	.1653	.1425	.3970	.3525		1.0465
	(.0385)	(.0344)	(.0344)	(.0326)	(.0317)	(.0319)	(.0287)	(.0287)		(.0365)
14L	.2113	.1050	.1040	.1810	.1762	.1565	.3485	.2927	.9572	
	(.0396)	(.0350)	(.0351)	(.0332)	(.0323)	(.0327)	(.0289)	(.0291)	(.0023)	

[a] r_G above diagonal; r_P below diagonal.

A relative efficiency greater than one indicates indirect selection would be more effective than selecting for trait Y directly. If we examine the respective values for four month weight and seven month smolt for the 1978 brood, a relative efficiency of .87 results. In this case, direct selection for seven month smolts would be more effective. Values for other traits for the 1977 and 1978 brood years are presented in Table 4. Examination of the results indicates there are only a few cases where indirect selection would be worthwhile and these are confined to freshwater rearing. Again, brood year differences are apparent. One conclusion that might be drawn is that early selection for freshwater traits will not apparently result in substantial gains over direct selection during seawater grow-out. These relationships must, however, be explored further before any definitive statements can be made. The results, nevertheless, are useful as first approximations.

Predicted Selection Gains

Heritability estimates (h^2), phenotypic standard deviations (σp), selection intensities (i), and the generation interval (L) were used to predict selection gains. The relationship can be written as gain = $h^2 i \sigma p / L$. The selection intensity could not be precisely estimated because of the varied types of selection actually practiced (index selection for families, phenotypic selection within select families, culling at pre- and post-seawater transfer) and the undetermined effects of pre-spawning mortality. Predicted gains were thus calculated using two estimates

Table 3. Genetic and phenotypic correlations among fresh- and seawater traits exhibited in progeny of the 1978 brood. One standard error is given in the parenthesis below each value.[a]

r_G / r_P	4W	5W	5L	7W	7L	7S	11W	11L
4W		.6671 (.2017)	.6633 (.1941)	.8240 (.2742)	.7712 (.2154)	.6687 (.3250)	.4403 (.3085)	.4381 (.3001)
5W	.4298 (.0417)		.9716 (.0200)	.9761 (.1487)	.8529 (.1208)	.7069 (.2210)	.4537 (.2721)	.3066 (.3021)
5L	.4638 (.0432)	.9487 (.0037)		1.0102 (.1719)	.9234 (.0994)	.7718 (.1983)	.5631 (.2294)	.4353 (.2600)
7W	.3851 (.0408)	.5108 (.0421)	.5654 (.0434)		.9857 (.0322)	.7808 (.1947)	.5095 (.1391)	.4081 (.1326)
7L	.4224 (.0418)	.5190 (.0433)	.5995 (.0449)	.9753 (.0021)		.9245 (.0733)	.6792 (.1541)	.6029 (.2309)
7S				.8408 (.0095)	.8681 (.0077)		.8718 (.1327)	.7528 (.1840)
11W	.2527 (.0388)	.2974 (.0391)	.3755 (.0409)	.1798 (.0391)	.1729 (.0404)			.9822 (.0146)
11L	.2741 (.0405)	.2427 (.0407)	.3354 (.0424)	.2328 (.0405)	.2351 (.0418)		.9614 (.0025)	

[a] r_G above diagonal; r_P below diagonal.

Table 4. Relative efficiencies of indirect over direct selection where traits X and Y are the traits directly and indirectly selected for, respectively. No efficiencies reported where $r_G < 0$ or $r_G > 1$. 1977 data above dashed line and 1978 data below dashed line.

	Trait Y								
Trait X	5W	5L	7W	7L	7S	11W	11L	15W	15L
4W	1.02	1.36	.97	1.22	.23			.70	.93
	.58	.52	.95	.75	.87	.47	.42		
5W		1.07	.30	.96				.19	.37
		.88	1.30	.96	1.05	.56	.48		
5L			0	.17				.24	.39
				1.14	1.27	.77	.53		
7W					.90			.63	.68
				.83	.87	.47	.34		
7L					.82			.78	.65
					1.23	.75	.60		
7S						.27	.18	.45	.55
						.72	.56		

for i; one estimate yields the maximum change possible, the other, the minimal change expected. Predicted gains for the progeny of BY 1977 and BY 1978 are presented in Tables 5 and 6 respectively. In almost all cases, even the conservative calculations predict gains in excess of 1 percent per generation. Such increases, if realized, will be substantially higher than those commonly expected for domestic livestock, indicating that the potential for genetic change in these two groups of coho salmon is substantial.

Table 5. Prediction of selection gains per generation (1977 Brood).

Trait		X	Max. Estimate	%	Min. Estimate	%
7						
Month	Weight	14.46 g	6.61 g[a]	46	.2 g[d]	1.4
	Length	101.45 mm	11.63 mm[a]	11	.6 mm[d]	.6
11						
Month	Weight	136.71 g	27.79 g[b]	20	3.3 g[d]	2.4
	Length	210.42 mm	28.97 mm[b]	14	.7 mm[d]	.3
14						
Month	Weight	374.17 g	72.00 g[c]	19	7.9 g[d]	2.1
	Length	305.37 mm	21.76 mm[c]	7	3.2 mm[d]	1.0

[a]Based on selecting 60 individuals out of 10,000.
[b]Based on selecting 60 individuals out of 7,000.
[c]Based on selecting 60 individuals out of 3,000.
[d]Based on index results and selecting 10 families out of 40.

Table 6. Prediction of selection gains per generation (1978 Brood).

Trait		X	Max. Estimate	%	Min. Estimate	%
7						
Month	Weight	17.63 g	7.53 g[a]	43	0.5 g[c]	3
	Length	107.49 mm	22.45 mm[a]	21	2.0 mm[c]	2
11						
Month	Weight	131.08 g	74.02 g[b]	56	9.5 g[c]	7
	Length	210.42 mm	63.14 mm[b]	30	7.0 mm[c]	3

[a]Based on selecting 60 individuals out of 10,000.
[b]Based on selecting 60 individuals out of 7,000.
[c]Based on index results and selecting 10 families out of 40.

We have preliminary indications that the selection techniques employed thus far have been very effective. Data on the freshwater growth of progeny resulting from the selected 1977 BY adults show that with an equivalent number of temperature units [$\Sigma(F^{o}-32)$/day], the selected progeny have outpaced their parents by a significant margin of 98 percent by the end of freshwater rearing. As no control line was available due to facility limitations, the increase due to environmental improvements could not be determined. However, since no significant changes in the rearing environment between the two groups were made, a large portion of the increase may be attributed to genetic improvement.

Conclusions

The genetic basis for freshwater and seawater growth of two brood years of accelerated, pen-reared coho salmon have been established. On the basis of heritability estimates, selection intensities, and correlations among traits, the potential for genetic improvement appears considerable for both groups. A broodstock selection program has been designed and implemented on the basis of these results. Preliminary results indicate that the initial steps of the program have been selected.

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Redistribution of Coho Salmon Catch to Different Fisheries by Stock Transplants

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Introduction

The benefits generated by hatchery production of coho salmon depend on total stock produced, total catch and allotment of the catch between different fisheries. Partitioning between fisheries implies both geographical and social impacts related to where, and by whom, the fish are harvested. Since the economic value of salmon depends to a large degree on this partitioning it is desirable to control catch distribution in order to maximize benefits.

Control is presently exerted at a secondary level by regulation of the fisheries but this is complex, often controversial and inefficient. Also, motivation is stock management and conservation rather than maximization of economic indicators. Control at a more fundamental level, for example by producing smolts with favorable behavior patterns, is possible and may be practiced to supplement regulatory jurisdiction over the fisheries.

Coho stocks of different origins released from a single site may be captured at differential rates in various fisheries (Hager and Hopley, 1981). This suggests that marine migration routes and timing are at least partially a function of stock origin. On the other hand, catch distribution of fish within a coho population is influenced by release size and time from a hatchery (Buckley and Haw, personal communication) and by downstream timing of wild smolts (Fisheries and Oceans, Canada, unpublished data). Thus, it is possible to influence to some extent the allocation of hatchery produced coho between fisheries by stock selection, by fish culture strategy, and perhaps by a combination of these factors.

The relative importance of stock origin, or genotype, compared to the cultural and release environment as determinants of marine catch distribution is, however, unknown. This may constrain the potential distributional benefits of stock transplants.

Preliminary recovery data for coho released in 1977 from the Big Qualicum Hatchery on the east coast of Vancouver Island and from Capilano Hatchery in North Vancouver (Figure 1) including a group transplanted from Big Qualicum to Capilano suggest that catch distribution is influenced by both stock origin and the rearing/release site.

Methods

Three groups of 1975 brood coho — Big Qualicum production, Big Qualicum stock transplanted to Capilano Hatchery as eyed eggs, and Capilano production — were reared on OMP diet for release as smolts in 1977 (Table 1). In total, 248,000 smolts were adipose clipped and tagged using nine different binary codes. The Capilano stock was also left ventral clipped to facilitate identification of returning donor adults in 1978.

Table 1. Release data summary for Big Qualicum and Capilano 1975 brood coho.

Stock	Release Site	Size (g)	Date	CWT (Agency 2)	No. CWT & AD
Qualicum	Qualicum	25.8	May 5-18	10/3	90,520
Qualicum	Capilano	18.7	May 30-June 6	16/17-22	123,403
Capilano	Capilano	19.6	June 6	16/16, 12/7	34,083

Catch estimates for the three groups were based on preliminary data from the coastwide mark recovery program. Recoveries in the Canadian commercial fishery were expanded by actual catch to sample ratios. Canadian sport recoveries were expanded by a factor of five assuming an angler awareness rate of 20 percent. U.S. commercial recoveries were expanded by a factor of three. Catch data for release groups represented by multiple tag codes were combined for the present analysis. Minor changes in the results are anticipated when the recovery data are finalized.

Results

Estimated commercial and recreational catch in 1978 from the 248,000 marked smolts released was 38,523 for an overall contribution of 15.5 percent. Catch rates were 12.7 percent for Big Qualicum production, 14.5 percent for the Big Qualicum to Capilano transplant, and 26.9 percent for Capilano production (Table 2). Catch of age two jack coho in 1977 was minor (less than 5 percent of the total catch) so was not considered in the analysis.

Table 2. Estimated catch (pieces) of 1975 brood marked coho in 1978, by catch region and total catch as percent of release.

Catch Region	Big Qualicum Production	Big Qualicum Transplant	Capilano Production
Alaska	6	0	0
North Coast	54	0	0
Central Coast	1,905	534	126
West Coast Vancouver Island	1,624	1,229	454
Johnstone Strait	2,312	1,312	216
Georgia Strait Commercial	1,535	3,427	1,258
Georgia Strait Sport	3,695	9,530	6,270
Juan de Fuca Strait	65	311	95
Washington	285	1,536	744
TOTAL	11,481	17,879	9,163
Tagged Smolts Released	90,520	123,403	34,083
Estimated Catch (% of release)	12.7	14.5	26.9

In order to compare distribution of the different groups, regional catch data for each group were expressed as proportion of the total catch for that group in eight catch regions (Table 2, Figure 1). This was necessary since absolute catch rates at least partially reflect different rearing conditions. For example, the Capilano production group which survived to harvest at twice the rate of the other groups (Table 2) was reared at extremely low density. Experiments at Capilano Hatchery have shown a definite interrelationship between coho rearing density and ocean survival (Sandercock and Stone, in press).

Capilano production of native stock contributed heavily to the "inside" Georgia Strait fishery with over 82 percent of the catch in this region (Table 3). No fish were recovered in the most northerly catch regions of Alaska and north coast, B.C. There was a low recovery rate in the central coast and Johnstone Strait fisheries (3.8 percent of the total catch). A significant portion of the Capilano stock appears to migrate south from Georgia Strait as

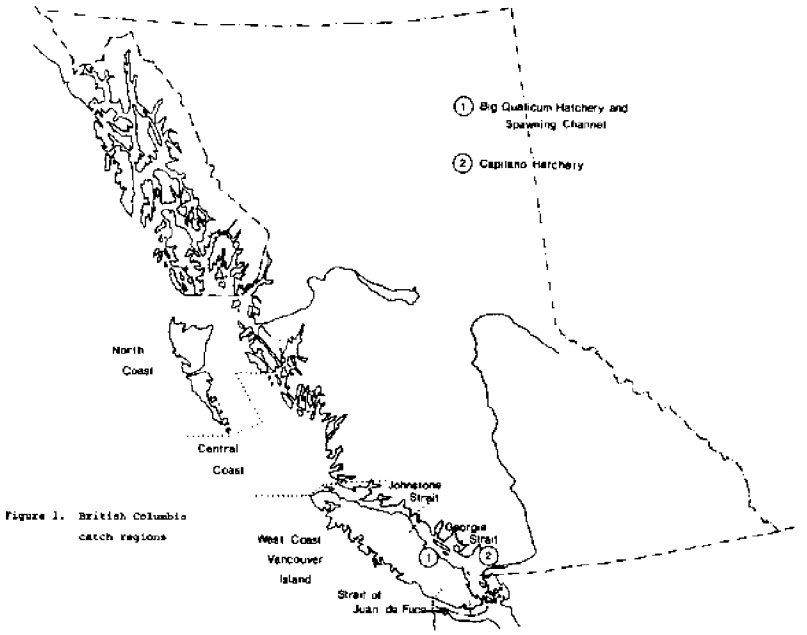

Figure 1. British Columbia catch regions.

Table 3. Proportion of catch of each stock by catch region (percent).

Catch Region	Big Qualicum Production	Big Qualicum Transplant	Capilano Production
Alaska	<0.1	0	0
North Coast	0.5	0	0
Central Coast	16.6	3.0	1.4
West Vancouver Island	14.2	6.9	4.9
Johnstone Strait	20.1	7.3	2.4
Georgia Strait	45.5	72.5	82.2
Juan de Fuca Strait	0.6	1.7	1.0
Washington	2.5	8.6	8.1
TOTAL	100.0	100.0	100.0

reflected by significant catches in Strait of Juan de Fuca and Washington coastal waters (9.1 percent of the total catch).

Big Qualicum hatchery production contributed more to outside fisheries than did Capilano production. Only 45.5 percent of the Big Qualicum catch was taken in Georgia Strait compared to 82.2 percent for Capilano production. Proportion of catch in the Johnstone Strait, central coast and west coast Vancouver Island regions in particular was much greater for Big Qualicum than Capilano production and Big Qualicum releases were the only fish recovered in the north coast and Alaska fisheries. Recoveries in the Strait of Juan de Fuca and Washington were relatively low.

The catch distribution of the transplanted Big Qualicum coho, released from Capilano, was intermediate between the catch patterns for the native stock production groups with the exception that this group had the highest percentage representation in the southern regions including Washington and Strait of Juan de Fuca. Georgia Strait catch amounted to 72.5 percent of the total and, like the Capilano production, none were recovered in the north coast or Alaska fisheries.

Discussion

It is recognized that catch distribution patterns may not necessarily reflect stock distribution due to differences in catchability and timing. However for the purpose of production planning, given established fishing effort, catch distribution is more critical than stock distribution. Certainly, migration timing may be an important factor in determining catch distribution of Capilano and Big Qualicum coho stocks considering that the Capilano stock is early migrating with peak escapement in late August whereas the Big Qualicum stock enters the river in late October and early November. This may account for some of the differences between Big Qualicum and Capilano production contributions to outside fisheries and between the Capilano production and Big Qualicum transplant group contributions to the Juan de Fuca and Washington fisheries.

Catch distribution of the Big Qualicum stock released from Capilano does not reflect that of either Big Qualicum or Capilano native stock production. The proportion caught in the central coast, west coast Vancouver Island and Johnstone Strait regions is greater than that for Capilano production but less than that for Big Qualicum production. Georgia Strait contribution is also intermediate between that for the two production release groups. Only in Strait of Juan de Fuca and Washington fisheries did the transplant group contribute at a rate outside of the range seen for the production releases.

The allotment of catch between marine commercial and sport fisheries further emphasizes the differences between the three release groups (Table 4). Approximately 68 percent of Big Qualicum production coho were taken by commercial gear. At the other extreme sports gear took 68 percent of the Capilano production. Catch of the transplanted stock was more equally shared by the two user groups with 46 percent taken in commercial fisheries and 54 percent taken in sport fisheries.

Table 4. Relative contribution of the production and transplant groups to the commercial and sport fisheries.

	Commercial (%)	Sport (%)
Big Qualicum	68	32
Transplant	46	54
Capilano	32	68

These results indicate that the catch distribution of the Big Qualicum coho transplanted to Capilano for incubation, rearing and release was influenced by both the genetic composition of the stock and the environmental history. This has significant repercussion for enhancement planning. If it is intended to improve hatchery contribution by transferring a stock with desired traits into a facility on a different watershed, it is apparent that the marine behavior of the transplant will be tempered toward that of the same species in the recipient stream. Thus, while resource managers have an opportunity to adjust the sharing of benefits from hatchery production by stock transplants, the potential for control may be limited by the influence of the freshwater environment on marine distribution.

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Section VI
Physiology of Smoltification

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Dissolved Oxygen: mg/l vs. pO_2 as a more Meaningful Indicator of Life Support for Fish in Aquaculture Systems

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Oxygen is a major constraint in fish culture. If oxygen levels are inadequate, fish growth is reduced, feed conversions increased, and feeding behavior altered (Davis, 1975).

Many investigators (Piper, 1970; Leitritz and Lewis, 1976; Westers and Pratt, 1977) have attempted to define minimum oxygen requirements of salmonids using the dissolved oxygen content (mg/l) of the water. However, the ability of fish to uptake oxygen is not directly dependent upon the dissolved oxygen content but is dependent upon the environmental partial pressure of oxygen (pO_2) (Figure 1). If environmental pO_2 is sufficiently high, the fish's rate of oxygen uptake is at a maximum and is independent of the environmental pO_2. This range of high pO_2 is termed the zone of respiratory independence. When environmental pO_2 drops below a critical level, termed the incipient lethal tension, the rate of oxygen uptake by fish is limited and is dependent upon the environmental pO_2. In this zone of respiratory dependence, fish growth and feed conversions are adversely affected. The oxygen partial pressure and the dissolved oxygen of saturated water depends upon the temperature and elevation of the water.

An increase in elevation from 0 to 5,000 ft. results in a 17 percent reduction of environmental pO_2. Dissolved oxygen is also reduced by 17 percent (Figure 2).

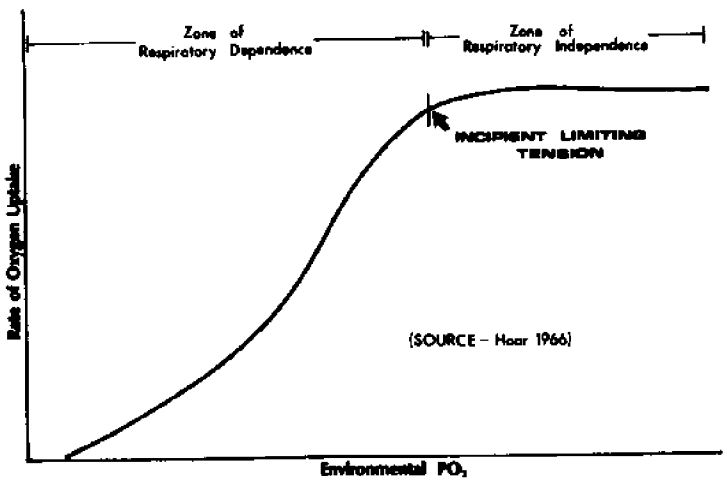

Figure 1. The effect of environmental partial pressure on the rate of oxygen uptake of fish.

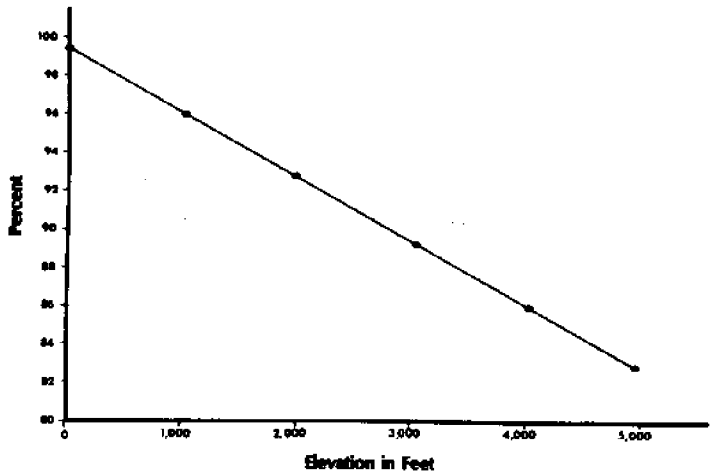

Figure 2. The relationship between elevation and the partial pressure of oxygen.

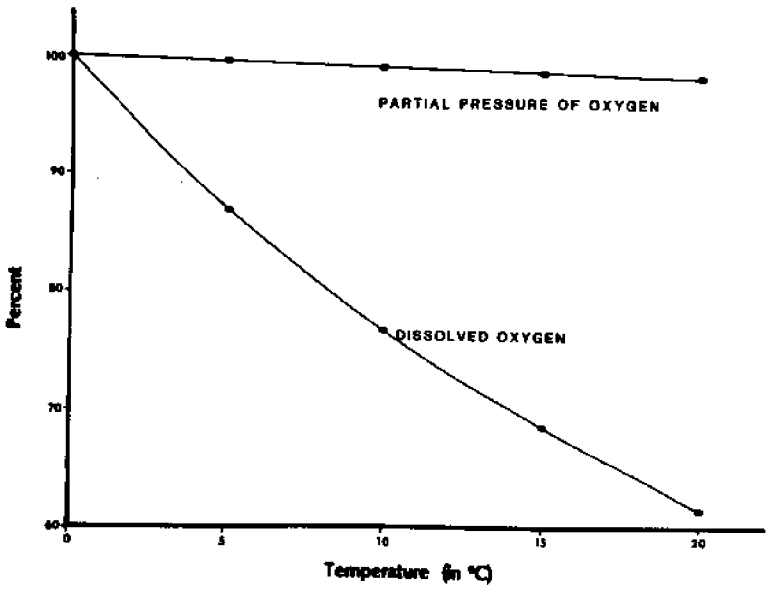

Figure 3. The effect of water temperature on the partial pressure of oxygen and dissolved oxygen.

Water temperature has a much greater influence on the dissolved oxygen than on the environmental pO_2 (Figure 3). When elevation is held constant and temperature increased from 0 to 20°C environmental pO_2 is reduced by approximately 2 percent. The same increase in temperature reduces the dissolved oxygen by 38 percent.

The relationship between constant dissolved oxygen (5 mg/l) and the environmental partial pressure it exerts at various temperatures is interesting (Figure 4). The environmental pO_2 of 5 mg/l of oxygen at 20°C is 86 mmHg. This is 160 percent environmental pO_2 exerted by 5 mg/l of dissolved oxygen at 0°C. Obviously, 5 mg/l of dissolved oxygen at different temperatures does not provide the same environmental pO_2 and, consequently, life support to fish.

It is apparent from the above discussion that the relationship between dissolved oxygen

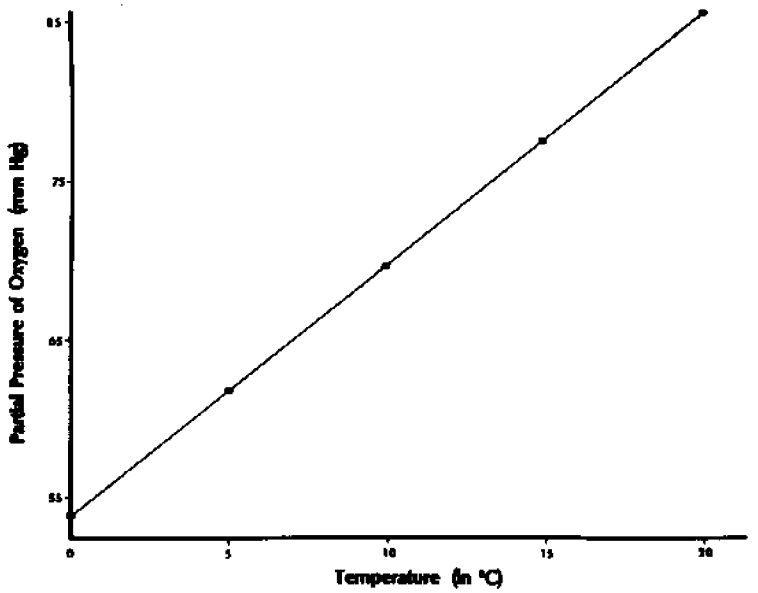

Figure 4. Oxygen partial pressure for 5 mg/l dissolved oxygen at various temperatures.

and environmental pO_2 is not constant and can be affected by water temperature. Since the rate of oxygen uptake is dependent upon the environmental pO_2, environmental pO_2 should be used in setting minimum oxygen limits for different elevations and temperatures. Although there has been much research conducted on the effects of enviromental pO_2 on blood-oxygen saturations, active metabolic rates, etc. (see Davis, 1975) relatively little work has been done on the effects of environmental pO_2 on fish growth. When this data becomes available, hatchery design criteria will be improved significantly.

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Present Situation and Some Problems of Marine Fish Propagation in Japan

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Abstract

Japanese high seas fishery production has greatly decreased since the enactment of a 200-mile economic zone by many countries. To counterbalance the impact of such a decrease, the Japanese government has started several extensive programs to develop aquaculture and coastal fisheries. These include development plans of hatching and releasing techniques, seed production, improvement of coastal aquafarms, construction of artificial fish reefs, sandy intertidal plains and others. A great deal of effort by national and prefectural institutions as well as fishermen's cooperatives makes it possible to manage large scale aquaculture of prawns, scallops, abalone and red sea bream. The culture of yellowtail and eel has also been established.

Along with an increase in aquaculture production, increased fish diseases, mass mortality and toxic fish and shellfish become a serious problem. These are primarily caused by water pollution, artificial and natural eutrophication.

This paper describes the present status of mariculture in Japan and discusses the related problems.

Introduction

The enactment of a 200-mile economic zone by many countries has had great impact on the Japanese high seas fishery. The yield has drastically decreased (Figure 1). Since the Japanese people take almost half of their animal protein from fishery products, it has become a serious imbalance of the former supply and demand.

Table 1 shows the prospect of supply and demand of fishery products in Japan in the years 1990 and 2000. It is apparent from the Table, that the fishery products will still maintain an important status as an animal protein source, and accordingly fishery products are expected to increase by 70 percent by the year 2000. The Japanese plan of fishery production development is given in Table 2. Figure 1 illustrates the expected trend of four types of fisheries. It is obvious that the offshore fishery and coastal fishery (including mariculture) steadily increase, while the high seas fishery is expected to continue to decline. It must be mentioned that the importation of fishery products will have a more important role in meeting the need of animal protein intake.

Present Status and Future Projects of Aquaculture

The techniques of culture and propagation have been well developed for several species of fish, crustaceans and shellfish. This is particularly true for sea bream, yellowtail, flat fish, Pacific salmon, scallops, oysters and abalone. The culture techniques have been established for seaweeds such as *Laminaria* and *Undaria*. These technical developments support a fairly large scale commercial mariculture industry.

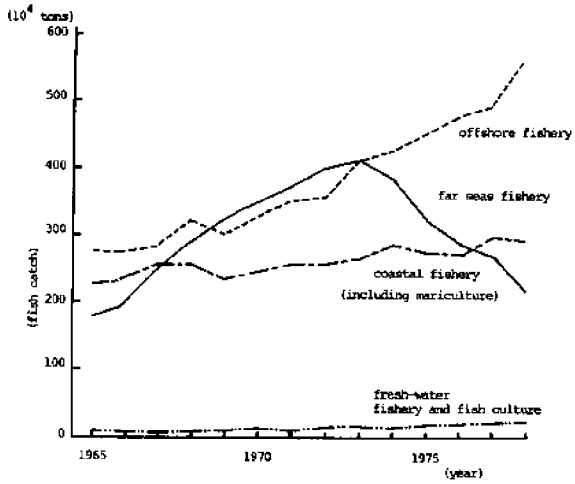

Figure 1. Trend in fish catch by the four groups of fishery operations in Japan (Anonymous, 1979b).

Table 1. Estimated demand for fisheries products in 1990 and 2000 (domestic demand, excluding seaweeds).

Item	Year	1976	1990 (1)	1990 (2)	2000 (1)	2000 (2)	Reference 1985
Per capita daily nutrition intake (g)	Total protein	79.4	85	85	88	88	83.3
	Animal protein	36.3	39.1	43.3	40.5	49.6	40.4
	Fish protein	17.9	19.2	21.2	19.8	24.3	20.6
Demand for fishery product (10,000 tons)	Food use	760.8	950	1,050	1,050	1,300	881.4
	Nonfood use	247.3	300	400	350	400	400.1
	Total	1,008.1	1,250	1,450	1,400	1,700	1,281.5
Fish for processing saved by effective utilization (10,000 tons)			50	50	100	100	
Demand for fishing product with fish for processing saved by effective utilization			1,200	1,400	1,300	1,600	
Population (ten thousands)			12,827		13,690		12,187

(Anonymous, 1979a).

Table 2. Japanese supply of marine productions in 1977 and projections of development in 1990 and 2000 (excluding seaweeds) (10,000 tons).

Item/Year	1976	1990	2000	Remarks
In 200 sea miles of Japan	605.1	850—1,050	900—1,200	including freshwater
High seas	41.7	50	100	including Antarctic Ocean
In 200 sea miles of foreign countries	350.6	200	200	
Net import (Import minus Export)	10.7 (113.6—102.9)	100 (150—50)	100 (150—50)	
Total amount of supply	1,008.1	1,200—1,400	1,300—1,600	

(Anonymous, 1979a).

Table 3 shows the trend of production of major species in aquaculture. From this table we know that the production has significantly increased since 1970. The increase in production of abalone, prawns and sea bream has greatly contributed to the development of aquaculture.

In 1976, the Japanese government started coastal fisheries development plans with a total budget of 200 million yen ($880,000). The primary purpose of the plan was to improve the fishery grounds for aquaculture. In 1978, a total of 577 artificial fish reefs were constructed,

Table 3. Mariculture production by species (unit: ton).

Year	Fishes							Crustaceans and Octopus				
	Horse mackerel	Yellow jack	Yellow-tails	Bream	Puffer	File fish	Others	Rock lobster	Kuruma prawn	Blue crab	Octopus	Others
1970	* 7	—	43,354	454	23	62	11	—	301	0	109	94
1971	* 57	—	61,855	930	15	18	38	—	306	1	98	339
1972	*127	—	77,059	1,380	14	39	104	—	454	1	68	1,118
1973	*387	—	80,439	2,741	16	40	150	—	659	0	56	4,675
1974	619	48	92,946	3,298	8	25	140	—	912	5	54	5,036
1975	920	22	92,407	4,462	9	8	170	—	936	—	41	6,313
1976	704	58	101,786	6,572	11	2	125	—	1,042	—	42	8,390
1977	743	161	115,098	8,245	15	10	238	0	1,124	—	16	7,463
1978	809	177	121,956	11,315	47	3	701	—	1,184	—	11	5,759

Year	Shellfish			Marine algae				Cultured pearl	Total
	Scallop	Oyster (with shell)	Others	Kelp *Laminaria*	Laver *Undaria*	Laver *Porphyra*	Others		
1970	5,674	190,799	4	284	76,358	231,464	—	85	549,082
1971	10,361	193,846	5	665	95,155	244,946	—	49	608,684
1972	23,049	217,373	36	3,340	105,795	217,906	—	42	647,905
1973	39,297	229,899	288	7,681	113,211	311,410	—	34	790,974
1974	62,651	210,583	129	10,201	153,762	339,314	—	30	879,761
1975	70,313	201,173	114	15,759	101,937	278,127	—	30	772,741
1976	64,946	226,278	73	22,096	126,701	291,050	—	34	849,909
1977	83,213	212,779	92	27,260	125,798	279,031	64	39	861,389
1978	67,750	232,068	207	21,890	102,665	350,471	194	37	917,244

(*including Yellow jack) (Anonymous, 1979b).

87 fish culture centers were established, and 37 sites were chosen for special fishery ground protection plans. These numbers will be increased by the year this project terminates (1982). The second project will start shortly after the evaluation of the first plan is finished.

The fish culture centers have taken a lead role in developing the culture techniques and carrying out artificial fertilization and release of spawn. The fish culture centers were established in 35 different locations in 1975, compared to only nine centers in 1963. Two prefectural governments have their own fishery centers. Figure 2 shows the coverage of areas by these centers surrounding almost all the coastal regions of Japan.

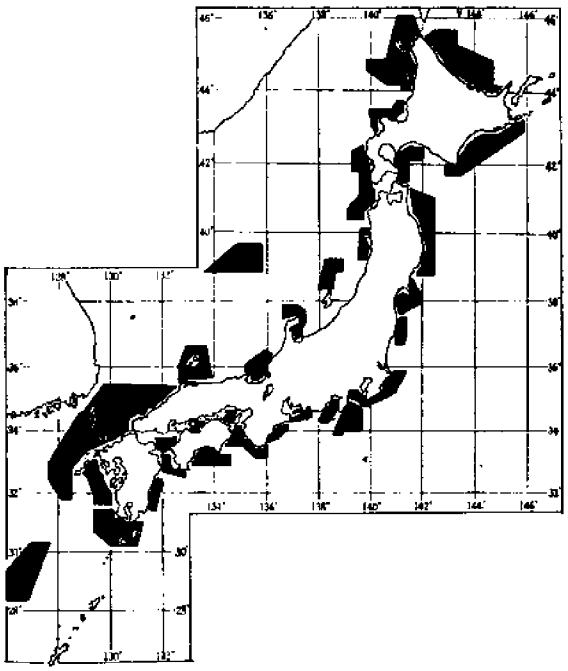

Figure 2. Designated sea area for promotion of development of fishery resources (Res. Inst. Ocean Economics, 1975).

Salmon propagation is one of the most important fish culture programs in Japan and has a long history. Currently the facilities and techniques make it possible to release a total of 850 million fry into 146 rivers in Hokkaido at the state-run hatcheries. A total of 470 million salmon fry have been released into 94 rivers in nine prefectures by fisheries cooperatives on Honshu. In 1979, the salmon propagation plan was greatly expanded to release salmon fry into 157 rivers.

The main salmon propagation plans in 1980 are as follows: 1) to conduct basic surveys on the suitable rivers for salmon stocking, 2) to improve the structure of rivers in order that returning salmon can avoid obstacles to their natural spawning grounds, 3) to improve the rearing techniques to facilitate the growth of salmon fry in confinement which in turn will decrease their mortality rate after they are released. The improvement plan of hatchery facilities belonging to the fishery cooperatives in Hokkaido and the production facilities for silver salmon on Honshu Island will also be strengthened in 1980.

Environmental Problems

Along with the advancement of fish culture and propagation, various accidental damage to fisheries has been occurring in the coastal regions of Japan. The main reason is believed to be the deterioration of the marine environment. Table 4 shows the increasing tendency of damage caused by red tides, pollution by waste oil and other contaminants. Mass mortalities

Table 4. Damage to fishery due to water pollution. Top figure: number of cases; bottom figure: amount of loss (million dollars, 1 $ = ¥ 226).

Case of damage/Year	1973	1974	1975	1976	1977	1978
Red water (discolored water)	27	30	26	27	36	41
	2.62	0.46	0.92	1.39	21.93	19.79
Pollution by waste oil	58	91	77	111	103	136
	7.82	114.28	11.93	5.54	9.22	16.41
Others	51	82	74	66	33	33
	6.28	8.42	3.18	7.54	0.28	0.47
Total cases of marine pollution	136	203	177	204	172	210
	16.72	123.16	16.03	14.47	31.43	36.67

(Anonymous, 1980).

of yellowtail and sea bream in culturing ponds in the Inland Sea have been frequently caused by red tides. In Hokkaido and Aomori prefectures, the occurrence of toxic plankton caused a serious economic loss to the scallop culturists. This resulted in a closure of the scallop market for a long period of time. Red tides have been reported frequently in recent years and tend to last longer than those in previous years.

Although water protection laws and related regulations have considerably prevented the deterioration of seawater by pollutants from factories and cities, oil waste water, poisonous materials, and thermal pollution have been reported to a considerable extent. The clarification of causes of fishery damage and assessment of environmental protection are urgent problems to be solved. Many national and prefectural government agencies and private institutions have been involved in the research on pollution and environmental protection.

Future Problems

Fish culture and propagation in Japan has been successful due to cooperation between fishermen, researchers and national and prefectural governments. However, there are many problems to be solved before attaining the above mentioned goal of increased fish culture and propagation.

Japan has a continental shelf of 440,000 km² which is equivalent to 1.2 times its land area. At present only 1/30 of the continental shelf has been utilized for fish culture and propagation (Table 5). An estimate indicates that at least half of the unused sea area can be utilized for mariculture. Since most of the potential area is under the influence of the open ocean, development of marine engineering is required to establish culturing facilities safe enough to resist adverse sea conditions. In particular, fish culture facilities and floating fish reefs in the offshore regions are needed. Advanced technology is also needed for marine environmental control systems, artificial seaweed farms, breakwater sites and intertidal sandy plains.

For the culture of fish such as salmon, red sea bream, prawns and abalone, the establishment of appropriate technology is needed to produce healthy spawn in large number. It is also necessary to select new culture species, especially in migratory fish such as tuna.

The most urgent need for fish culture is the establishment of the appropriate food for the different life stages of the animals. The future research plans will have to enlist the

Table 5. Land area and the area of economic zone (estimated area by water depth).

	Total	Land	0-200 m	200-500 m	500-1000 m	1000-6000 m	6000 m+
Area (10,000 km²)	488	37	44	15	23	347	22
Ratio (%)	100	7.7	9.1	3.1	4.6	71.7	4.4
Area in ratio compared with land area	133	1.0	1.2	0.4	0.6	9.4	0.6

(Res. Inst. Ocean Economics, 1975).

technology of efficient use of inland water. Ecosystem studies of the sea area where the release of the new culture species will occur are also needed. The prey-predator relationships and food availability for released fry in the sea must be intensively studied.

Reforms of law and finance will strongly support the promotion and introduction of the new aquaculture technology.

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Aug. 1980, Anchorage, Alaska

Growth and Smolting of Underyearling Coho Salmon in Relation to Photoperiod and Temperature

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Abstract

Coho fry were raised in 197-liter tanks at temperatures ranging from 8 to 18°C in freshwater, 10 parts per thousand (ppt), or 20 ppt salinity under simulated natural or artificial photoperiods for 20 weeks. Growth rates were determined by periodic weighing of all fish at three to four week intervals throughout the experiments. Samples of 12 fish were removed from each tank after weighing and transferred to seawater to determine the rate of development of hypoosmoregulatory capacity as a measure of smoltification.

Temperature and photoperiod had the greatest influence on growth and smolting. Growth was greatest at 17°C, but optimum hypoosmoregulatory capacity for coho reared in freshwater developed at 14°C. Under simulated natural photoperiod, the optimum date for transfer to seawater was 30 June. No optimum was discerned in the fish exposed to artificial photoperiods. Growth was also best under simulated natural photoperiod. Rearing in 10 or 20 ppt salinity facilitated adaptation to seawater only slightly and had little effect on growth.

Introduction

A recurring problem encountered in the culture of anadromous salmonids is to determine the optimum time for their transition from freshwater to seawater residence (Wedemeyer, et al., 1980). There is considerable evidence that early growth and development to the smolt phase in several species is controlled by the annual seasonal cycle of environmental factors such as temperature and photoperiod (Folmar and Dickhoff, 1980; Hoar, 1976; Wedemeyer, et al., 1980). Furthermore, it has been observed that smolting can occur within six months of hatching when coho fry are reared at elevated temperatures (Donaldson and Brannon, 1976; Garrison, 1971). However, there is little quantitative information concerning the combination of environmental conditions required to produce underyearling coho smolts.

The present experiments are part of a study of techniques for accelerating growth and advancing the time of smolting in juvenile coho salmon. Earlier experiments in this laboratory demonstrated that compression of the annual photoperiod cycle by advancing the solstice at a reduced amplitude of change of daylength was more stimulatory to growth and smolting than was merely accelerating the rate of change of daylength (Clarke, et al., 1981). Since full smolt development of coho was not attained in the earlier 12 week experiment, the present experiments were conducted for 20 weeks.

Materials and Methods

Coho salmon eggs were taken at the Big Qualicum River hatchery on Vancouver Island in November and transferred to the Pacific Biological Station for incubation. Fry were held in

197-liter tanks in five laboratory rooms, each with independent light control (Clarke, et al., 1978). Laboratory conditions and sampling procedures were as described previously (Clarke, et al., 1978 and 1981).

In Experiment I, fry were randomly distributed among 29 tanks at a weight of 0.4 g. The experiment consisted of a complete factorial of three temperatures (8, 11.5, 15°C), three salinities (freshwater, 10 ppt, 20 ppt) and three photoperiods (simulated natural, accelerated, and reduced amplitude) for a total of 27 groups of 60 fry each. Two additional freshwater tanks were set at 8 and 15°C under a constant 17 hour daylength. The photoperiod regimes are illustrated in Figure 1. Acclimation to temperature was completed during the second week of the experiment and to salinity, during the third week. Fish were fed to excess with Oregon Moist Pellets using automatic feeders. The length of the daily feeding period was the same for all photoperiod treatments in order to avoid confounding the effects of photoperiod on appetite with those on feeding opportunity.

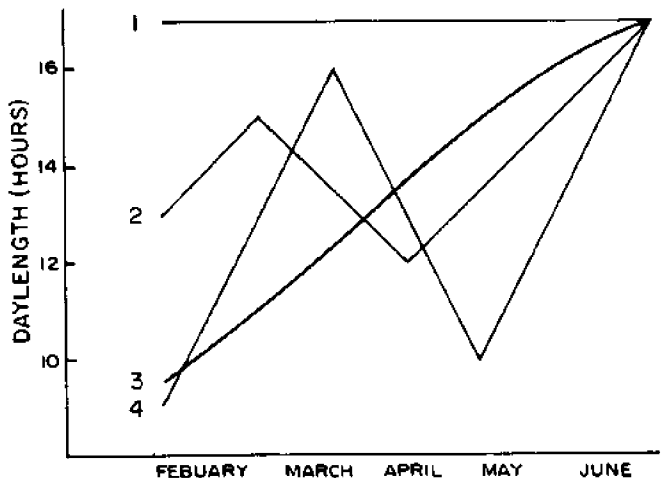

Figure 1. Schedule of daylength manipulation for: 1) constant 17 hour photoperiod; 2) reduced amplitude photoperiod; 3) simulated natural photoperiod; 4) accelerated photoperiod. The dynamic photoperiods were achieved by manual adjustments of the timers twice weekly to produce the appropriate increase or decrease.

Experiment II was conducted in the following spring. One hundred coho fry were weighed into each of five tanks at a size of 0.4 g and reared in freshwater at temperatures of 8, 11, 14, 17, and 18°C until mid-July under simulated natural photoperiod. Oregon Moist Pellets were presented in excess of satiation during four 30-minute periods daily, using automatic feeders. The four meals were spaced equally over the daylight period, increasing from 11 hours at the beginning to 17 hours at the end of the experiment.

In both experiments, all fry in each tank were weighed individually at three to four week intervals. In addition, the development of hypoosmoregulatory capacity was assessed by taking samples of 10 to 12 fish from each tank and subjecting them to a 24 hour seawater challenge test (Clarke and Blackburn, 1977).

At the end of Experiment II, seven treatment groups representing a range of performance in the final seawater challenge test were selected for a study of growth rate in seawater. Thirty fish representing the medium-sized individuals were selected from each of the groups and placed in tanks of seawater (29 ppt). Growth performance was assessed for 40 days.

The growth data from Experiment I were analyzed using factorial analysis of variance. The data from the seawater challenge tests were subjected to nonlinear response surface analysis (Lindsey, et al., 1970; Lindsey and Sandnes, 1972) in order to generate isopleths of plasma sodium in relation to rearing conditions.

Results

Experiment I

Growth

The factors with the greatest influence on growth were temperature and photoperiod (Figure 2). Analysis of variance on weight at the end of the experiment indicated that these effects were statistically significant ($p < .005$ and $p < .05$, respectively). Although growth appeared to decline slightly at 20 ppt, this effect was not statistically significant. There was no significant interaction among the effects of temperature, photoperiod, and salinity on growth.

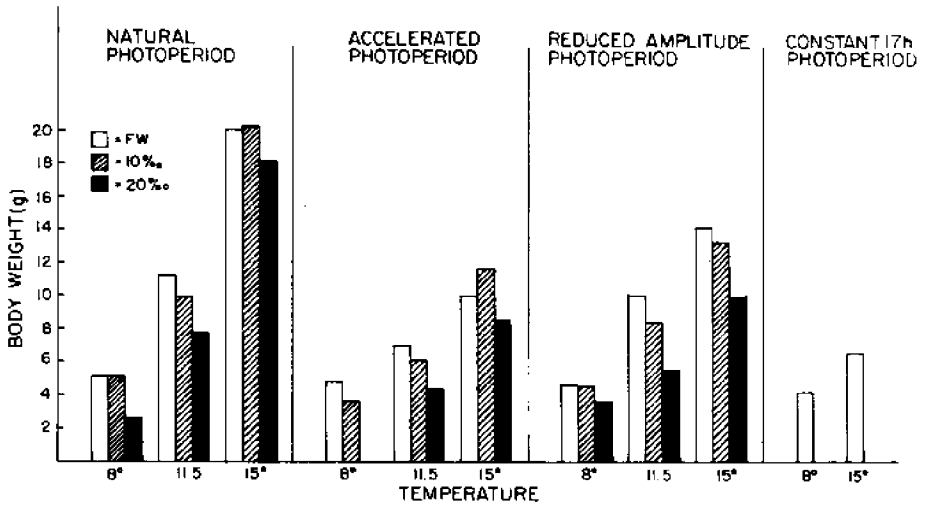

Figure 2. Mean body weight achieved in each tank at the end of Experiment I in relation to temperature, photoperiod, and salinity. Initial weight of the fry was 0.4 g.

Mortality

The rate of mortality was less than 5 percent in most tanks. However, the 20 ppt, 8°C tank under accelerated photoperiod suffered 50 percent mortality, most of which occurred between weeks 13 and 15 of the experiment. No disease agent could be identified and the deaths were attributed to osmoregulatory failure. The mortality, coupled with an accidental loss of fry and removal of samples for seawater challenge, resulted in an absence of fish in this treatment group during the last four weeks of the experiment.

Smolting

All three environmental factors influenced the ability of coho to adapt to seawater. Performance in the seawater challenge test was improved with increasing temperature and salinity. The artificial photoperiod treatments all suppressed smolting in comparison with the simulated natural photoperiod. The magnitude of this effect is evident from a comparison of the mean plasma sodium concentration after seawater challenge in samples taken from the freshwater, 15°C tanks at the end of the experiment: natural photoperiod, 155.3; reduced amplitude photoperiod, 160.3; accelerated photoperiod, 179.4; 17 hour constant photoperiod,

202.5 meq/L. Response surface analysis failed to obtain an optimum within the test space for fish reared under the reduced amplitude and accelerated photoperiods. However, analysis of data from groups reared under simulated natural photoperiod indicated an optimum of 153.9 meq/L sodium at 4.8 ppt salinity, 13.9°C on 18 June. The relative likelihood function for the response center coordinates was > 0.1 (i.e., comparable with a normal 95 percent probability interval; Lindsey, 1970) for salinities of 0 to 14 ppt, temperatures of 13 to 15°C, and seawater transfer dates of 9 June to 14 July.

Experiment II

Growth

From an initial size of 0.4 g, the mean wet weight achieved by mid-July increased from 8.5 g at 8°C to 39.7 g at 17°C and then dropped sharply to 22.2 g at 18°C (Figure 3). These weights are somewhat greater than those obtained at comparable temperatures in Experiment I, due to the longer daily feeding period.

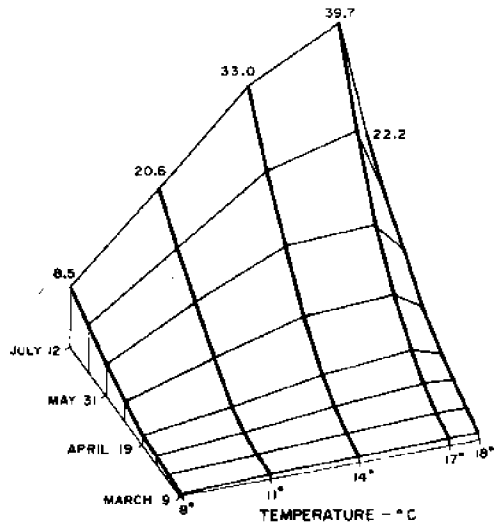

Figure 3. Effect of temperature on growth in Experiment II. The heavy lines represent the growth curves at each temperature from the end of the acclimation period in early March through 12 July. The numbers at the end of each growth curve indicate the mean body weight in grams at the end of the experiment.

Mortality

Eight fish died in the 8°C tank, four in each of the 11, 14, and 17°C tanks and six in the 18°C tank.

Smolting

Performance in the seawater challenge tests improved from early May through June and then declined in July. Response surface analysis of data from groups under natural photoperiod in Experiment I combined with the data from Experiment II indicated an optimum of 154.3 meq/L sodium at 14°C on 30 June for fish reared in freshwater (Figure 4). Inclusion of salinity as a factor in the analysis advanced the optimum transfer date by four days. The predicted center of 154.6 meq/L sodium now occurred at 0.5 ppt salinity, 14.5°C on 26 June. The relative likelihood function for the center coordinates was > 0.1 for salinities of 0 to 2.4 ppt, temperatures of 14 to 15°C and transfer dates of 26 June to 27 June.

Growth in Seawater

During the 40 day period in 29 ppt seawater, mean specific growth rate was inversely

related to mean plasma sodium concentration at the time of transfer, ranging from 1.17 percent per day to 0.31 percent per day. The relationship was described by the following regression equation:

$$G = 6.76 - 0.034 \times Na$$

where G is specific growth rate and Na is plasma sodium concentration after 24 hour seawater challenge ($R^2 = 0.79$, $p < .01$).

Discussion

The results of the present experiments indicate that photoperiod and temperature are the most important factors influencing growth and smolting of underyearling coho salmon. The interplay of temperature and photoperiod on hypoosmoregulatory capacity is readily seen from examination of the response surface (Figure 4). During May, performance in the seawater challenge tests were influenced strongly by temperature, with the greatest hypoosmoregulatory capacity observed in fish reared at temperatures of 11 to 15°C. At the time of optimum at the end of June, the influence of temperature was less marked. By July, performance declined rapidly regardless of temperature, suggesting that photoperiod is the primary cue for reversion to the parr condition. The center of the response surface was synchronized by the natural photoperiod cycle, since none was observed in the groups exposed to artificial photoperiods.

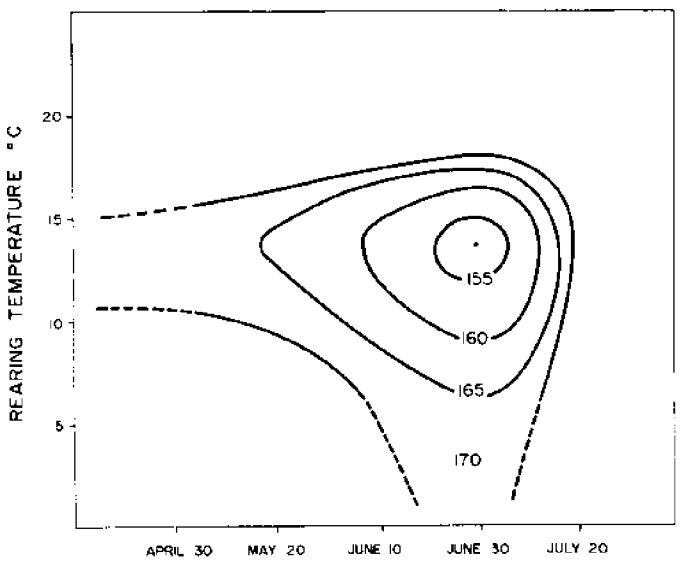

Figure 4. Effect of temperature history and time of transfer to seawater on plasma sodium concentration in a 24 hour seawater challenge test of underyearling coho reared in freshwater. Isopleths are drawn for 155, 160, 165, and 170 meq/l sodium. The center point represents 154.3 meq/L sodium at 14°C on 30 June.

The results of the present experiments are in accord with our previous finding that compression of the annual photoperiod cycle by advancing the solstice while maintaining the normal rate of change of daylength (i.e., reduced amplitude) is more stimulatory to growth and smolting than is accelerating the rate of change of daylength (Clarke, et al., 1981). However, these photoperiod regimes failed to accelerate growth and smolting in comparison with a natural photoperiod cycle. On the other hand, experiments with Atlantic salmon and steelhead trout parr during fall and winter have resulted in more rapid growth and advanced

smolting under accelerated or phase-advanced photoperiods (Knutsson and Grav, 1976; Komourdjian, et al., 1976; Saunders and Henderson, 1970; Wagner, 1974). It would seem that underyearling coho require elevated temperatures to grow to smolt size and that they are incapable of responding to the usual techniques of photoperiod acceleration during spring and early summer when growth is already rapid. The constant 17 hour photoperiod regime strongly suppressed both growth and smolting, indicating that changing daylength is a necessary cue for synchronization of normal growth and development.

The intermediate salinities used in Experiment I had a relatively slight influence on growth and smolting. A salinity of 20 ppt depressed growth only slightly, except under the accelerated photoperiod at 8°C where it caused heavy mortality. The slight growth suppression under natural photoperiod is not altogether surprising, since the fry were introduced to 20 ppt salinity at a size of only 0.5 to 1.0 g. Transfer of larger presmolt coho to 20 ppt salinity would not be expected to have any effect on growth (Clarke, et al., 1981). However, salinities of 29 to 30 ppt cause growth stunting in presmolt coho (Bern, 1978; Clarke and Nagahama, 1977). Rearing coho in 10 or 20 ppt salinity improved performance in the seawater challenge test only slightly. This minor effect does not seem to justify a substantial capital investment to incorporate seawater into rearing systems for presmolt coho. Nevertheless, if the use of brackish water is advantageous for other reasons (see Clarke, et al., 1981), the present results indicate that it can be used successfully.

The predicted maximum hypoosmoregulatory capacity for coho reared in freshwater occurred at 14°C on 30 June. Inspection of the growth curves (Figure 3) reveals that the mean wet weight at 14°C on this date is 27.5 g. This corresponds rather closely to the center of the surface for maximum return of adult coho obtained by Bilton, et al. (1980). They estimated maximum returns from release of smolts at a mean weight of 25 g on 22 June. A plausible hypothesis for this correlation between hypoosmoregulatory capacity and marine survival was postulated by Houston (1959, 1961). He observed that locomotor performance of chum fry was depressed during adaptation to seawater, coincident with the process of osmoregulatory adjustment (Houston, 1959). He suggested that as a result, smolts may be more vulnerable to predation during this period and ". . .release of hatchery fish at the weight and length producing most efficient adaptation to sea water should reduce both the extent and duration of adjustive changes and hence susceptibility to predation" (Houston, 1961).

In addition to this short-term effect on locomotor activity, the 40-day growth test in seawater at the end of Experiment II revealed a prolonged effect of the rate of adaptation to seawater on subsequent ability to grow. Groups of coho which had the greatest decline in hypoosmoregulatory capacity in July also grew least when held in seawater. For smolts released into the natural environment, slow growth would likely lead indirectly to higher mortality through predation.

Experimental releases of underyearling coho have usually produced very low rates of adult return in comparison with normal yearling smolts (see Bilton and Jenkinson, 1980). The coordinates for optimum performance of underyearling coho in the seawater challenge test indicate that the release "window" is more restricted for these accelerated fish than for normal yearling smolts. This makes it much more difficult to release underyearling coho smolts at the correct time. Reduced viability during marine life might also occur as a result of delayed effects of acceleration on adult metabolism and maturation. Donaldson and Brannon (1976) observed an increase in the rate of return of two-year-old adults from release of six-month coho smolts over a period of several years. They suggested that a special stock was being selected, since the accelerated smolts produced in successive years were the progeny of two-year-old adults.

Acknowledgements

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Thyroid Hormones in Smoltification of Anadromous Salmonids

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Abstract

Analysis of plasma concentrations of thyroxine (T_4) and triiodothyronine (T_3) of smoltifying yearling coho salmon maintained at ten Columbia River hatcheries has revealed a distinct springtime peak in plasma T_4. Groups of these hatchery-reared fish were transferred to seawater net-pens at the aquaculture facilities at Manchester, Washington at times corresponding to their hatchery release dates. Data regarding the percentage of surviving and smolted fish were collected throughout six months of seawater residence. These data were compared with various parameters of the freshwater T_4 peak. One aspect of the T_4 peak (proportion of the area under the T_4 curve) showed a significant correlation with survival of fish in seawater after six months. These data suggest that analysis of plasma T_4 of smolts in freshwater may provide a predictive index of their seawater performance. Similar studies comparing yearling coho salmon with underyearling ("O-age") fish whose growth was accelerated by rearing at elevated water temperature indicated a seawater performance curve correlated with a T_3 peak which occurred during the early summer. These results suggest that smoltification of yearling and O-age salmon occurs by different mechanisms. These data suggest that analysis of changes in plasma thyroid hormones provides useful information regarding the optimal time to transfer cultured salmonids from fresh- to seawater facilities.

Introduction

Since the first observations of the activation of the thyroid gland during salmon smoltification (Hoar, 1939), the thyroid has been implicated in a variety of biochemical, physiological and behavioral changes associated with the parr-smolt transformation (see reviews by Dodd and Matty, 1964; Fontaine, 1975; Woodhead, 1975; Hoar, 1976; Bern, 1978, Wedemeyer, et al., 1980; Folmar and Dickhoff, 1980a). Recently, through the use of modern biochemical techniques, principally hormone radioimmunoassay, seasonal cycles of plasma thyroid hormone concentration have been quantified in brook trout, *Salvelinus fontinalis* (White and Henderson, 1977), rainbow trout, *Salmo gairdneri* (Osborn, et al., 1978), coho salmon *Oncorhynchus kisutch* (Dickhoff, et al., 1978) and masu salmon, *Oncorhynchus masou* (Nishikawa, et al., 1979). Evidence accumulated to date indicates that the plasma concentration of thyroid hormones during smoltification of coho salmon is influenced by a range of genetic and environmental (photoperiod, temperature, diet, xenobiotic, etc.) factors. Thus, measurement of thyroid hormones of salmon appears to be a sensitive biochemical index of those factors which may influence parr-smolt.

Additional studies of the cycles of thyroid hormones in coho salmon with relation to the time of seawater entry of particular stocks of fish have suggested that such measurements may be useful indicators of the extent of smoltification in freshwater (Folmar and Dickhoff, 1980b). An accurate, predictive index of the appropriate time for transfer of salmon to

seawater is of obvious value to salmon aquaculturists. The present report discusses the relationship between thyroid hormones, time of seawater entry and survival of salmon in seawater net-pens in both yearling and 0+ age coho salmon.

Materials and Methods

Yearling coho salmon used in this study were obtained from hatcheries located in the Columbia River basin. Plasma samples were taken from fish in freshwater at two-week intervals from March through June. Samples were stored frozen until they were assayed for thyroid hormone concentration. At times corresponding to the release dates at the various hatcheries, approximately 650 fish were collected from each hatchery and transported by tank truck to the National Marine Fisheries Service Aquaculture Research Station at Manchester, Washington. Upon arrival at Manchester, the fish were placed in freshwater, weighed and measured, and then divided into two groups: one for destructive subsampling (350 fish) and the other for a six-month growth and survival study (300 fish). Fish designated for the survival study were further divided into two groups of 150 fish each; one group was vaccinated with a bivalent *Vibrio* vaccine, and the other was sham injected and fin clipped. Vaccination was accomplished by a 0.15 ml intraperitoneal (ip) injection of heat killed *Vibrio anguillarum* (Biotype I and Biotype II in a 3:1 ratio; Schiewe, et al., 1977) in a vehicle of 0.85 percent sodium chloride solution. At this point, all of the experimental fish were transferred to seawater net-pens (2.1 x 1.2 x 1.2 m nylon mesh with 5 mm aperture, Delta weave). At the time of transfer, the salinity of Clam Bay was 27 parts per thousand (ppt). During their six months in seawater, all fish were weighed to the nearest 0.1 g and measured to the nearest mm at approximately 30-day intervals from May through November. Mortalities were removed from the net-pens twice daily.

For the study comparing 0-age and yearlings, experimental fish were obtained as eggs from the Toutle River hatchery (Washington Department of Fisheries) in November of 1977. The eggs were transported to the National Marine Fisheries Service, Northwest and Alaska Fisheries Center in Seattle, and then divided into two test groups; one group was placed in an accelerated growth regime to enter seawater as 0-age animals (1978), while the other group was reared under normal hatchery conditions to enter seawater as yearling animals (1979). Acceleration of growth was accomplished by increasing the water temperature in 1°C/day increments, from 8 to 12°C at the swim-up stage and then maintaining the temperature at 12 to 13°C until transfer to seawater. The fish reared for transfer to seawater as yearlings were maintained on an ambient temperature regime which ranged from 6°C in the winter months to 20°C in late summer.

While in freshwater, the yearling group experienced epizootics of both myxobacterial disease (*Myxobacterium* sp.) in 1978 and furunculosis (*Aeromonas salmonicida*) in 1979. The outbreak of furunculosis occurred concurrently with the fifth serial seawater entry of the yearling fish. These disease outbreaks were treated with the antibiotic, chloramphenicol, which was administered in the diet at 3 g/kg of food for a ten-day period.

Sampling to collect plasma from fish to freshwater was commenced in April 1978 for the 0-age fish and March 1979 for the yearling fish and continued at two-week intervals throughout the freshwater residence period. The 0-age fish were transferred to seawater net-pens on 15 May and then every two weeks thereafter throughout August 1978 (eight entries). The yearling fish were transferred to seawater net-pens at two-week intervals from 15 March through 15 June and then at one-month intervals through 15 September 1979 (nine entries).

For the blood sampling, fish were stunned by a blow to the head and the tail was severed. Blood was collected from the caudal vessels with a heparinized pipette. Blood was centrifuged to separate the plasma. Plasma samples were stored frozen until assayed. Plasma con-

centrations of thyroxine (T_4) and triiodothyronine (T_3) were measured using specific radioimmunoassay methods (Dickhoff, et al., 1978). Antisera were purchased from Endocrine Sciences (Tarzanna, Calif.); I^{125}-labeled hormones (specific activity: 750 to 1300 mCi/mmole) were obtained from New England Nuclear (Boston, Mass.). A 10 μl volume of plasma sample (unknown) or standard containing known concentrations of thyroid hormone diluted in hormone-free salmon plasma was dispensed into 12 x 75 mm glass test tubes. Standards were run in triplicate; samples were run in duplicate. To each of the assay tubes, 250 μl of the following mixture was added: bovine γ-globulin (Cohn fraction II, Sigma), 100 mg; 8-anilino-1-naphthalenesulfonic acid (sodium salt), 60 mg; 8 x 10^6 cpm radiolabeled hormone; 0.11 M barbital buffer (pH 9.0), 100 ml; antiserum. The amount of antiserum added was adjusted to result in 50 percent radiolabeled hormone bound with no added unlabeled hormone. The standard curve ranged from 2.5ng/ml to 40 ng/ml of T_4 or T_3. Assay tubes were capped and incubated for two hours at 37° followed by 15 minutes at 4°. Antibody was then precipitated by addition of 0.3 ml of cold (4°) 20 percent (w/v) polyethylene glycol followed by thorough mixing. The precipitate was centrifuged at 2000 g for 15 minutes at 4°.The supernatant was decanted by inverting the tubes. The pellet was counted in a Micromedic gamma well counter for three minutes per tube.

The standard curve was constructed and unknowns were calculated by a log-logit plot and weighted regression analysis (Rodbard, 1974) using a Hewlett-Packard 9815A computer.

Results

A typical pattern of change in plasma concentrations of T_4 and T_3 of yearling coho salmon in freshwater is shown in Figure 1. Plasma levels of T_4 showed a distinct peak while the concentration of T_3 showed either a gradual increase or no change. The peak height and duration of elevated plasma T_4 concentrations were unique for each hatchery stock. However, the time of highest T_4 concentration was similar for all groups sampled in 1978.

The effect of transferring fish from freshwater to seawater is shown in Figure 2. Within one to four days of seawater residence, the plasma concentration of T_4 fell to a level equivalent to the lowest measured in freshwater. Plasma concentrations of T_4 in fish remaining in freshwater stayed elevated (Figure 2, dotted line). Thus, the cycle of plasma T_4 was shorter for those fish entering seawater.

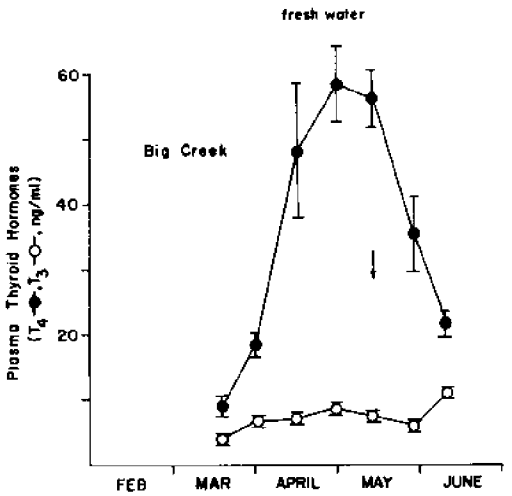

Figure 1. Plasma concentrations of thyroxine (T_4) and triiodothyronine (T_3) of yearling coho salmon during smoltification in freshwater at Big Creek hatchery.

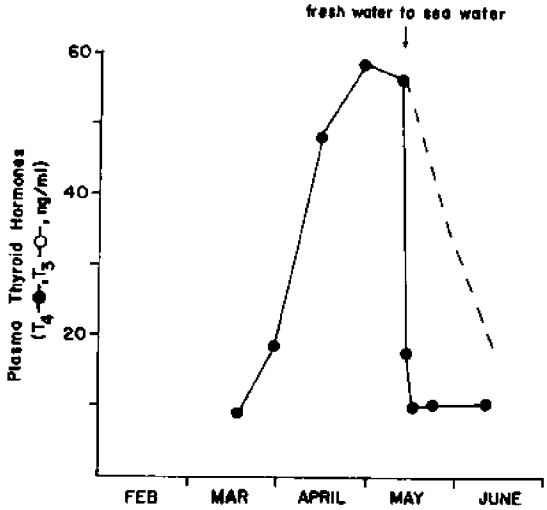

Figure 2. Effect of transferring coho salmon to seawater (arrow) on plasma T_4 and T_3 concentrations.

Various aspects of the cycles of T_4 in fish remaining in freshwater or entering sea water were compared with the survival of each hatchery stock of fish in seawater net-pens after six months. A significant relationship between survival and the proportion of the area under the T_4 peak at the time of seawater entry was found. This is explained in Figures 3 and 4. The proportion of the T_4 peak (percent T_4 peak) was determined by dividing the area under the T_4peak for the group of fish entering seawater (area A, Figure 3) by the area under the T_4 peak for the group remaining in freshwater (area A x area B, Figure 3). A plot of the precent of the T_4 peak versus percent survival of fish after six months in seawater net-pens is shown in Figure 4. This analysis implies that a greater proportion of fish will survive in seawater net-pens if they are transferred to seawater near the completion of the freshwater cycle of plasma T_4 concentration.

The patterns of change in plasma thyroid hormone concentrations of 0-age and yearling coho salmon in freshwater are shown in Figure 5. There were peaks of plasma T_4 concentra-

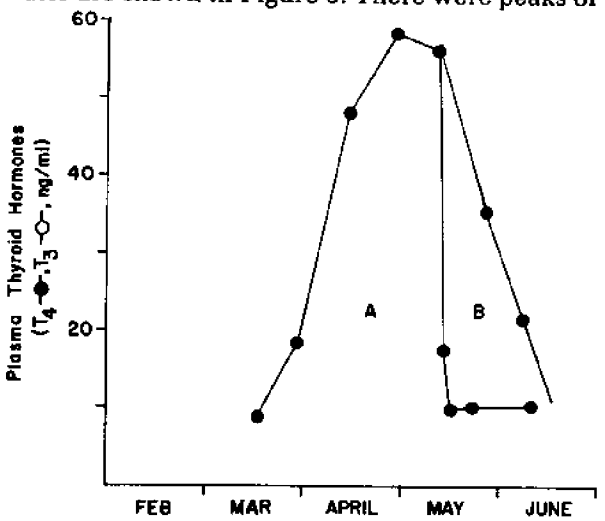

Figure 3. Analysis of patterns of change of plasma T_4 concentrations of yearling coho salmon remaining in freshwater and those transferred to seawater. The integrated area under the T_4 curve of fish entering seawater (A) was divided by the area under the curve for fish remaining in freshwater (A + B). This calculation resulted in a proportion of the T_4 curve (A/A + B) designated "% T_4 peak."

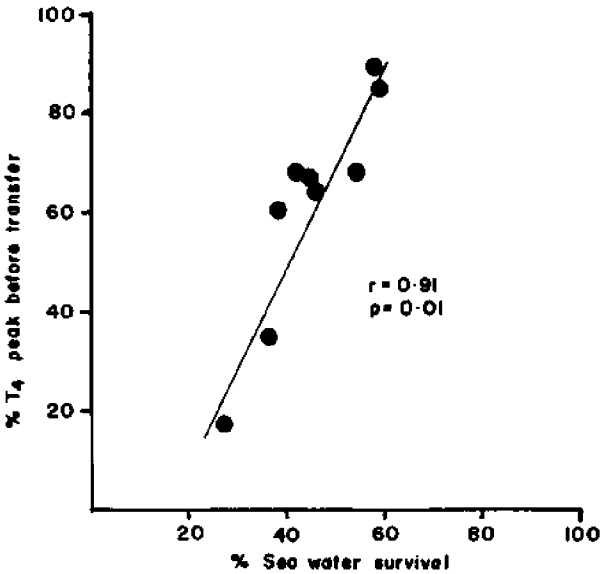

Figure 4. Relationship between percent T_4 peak and percent survival of yearling coho after 6 months of residence in seawater net-pens.

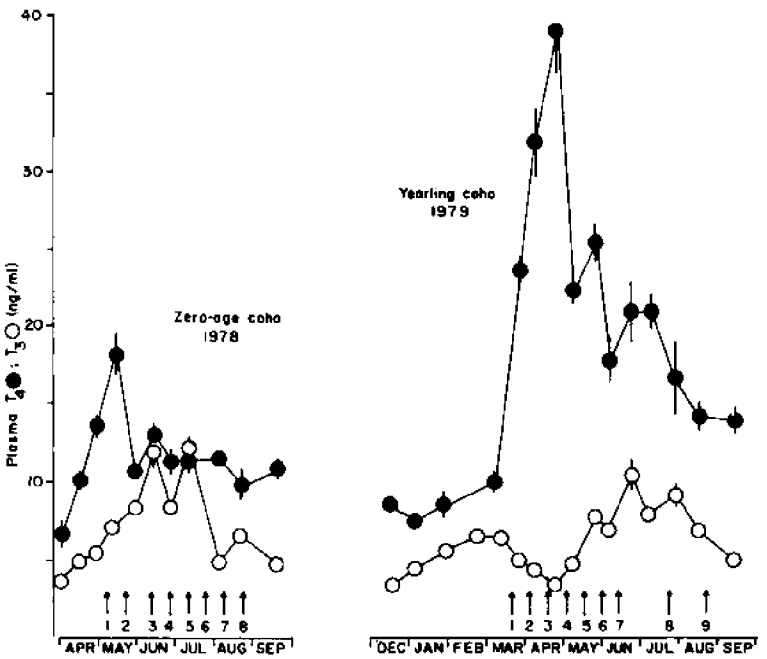

Figure 5. Change in plasma thyroid hormones of coho salmon during the first (1978) and second (1979) year of development in freshwater. Arrows indicate the time of transfer of groups of fish to seawater net-pens.

tion in both age groups. However, the yearlings had a T_4 peak which was greater in magnitude and occurred earlier in the spring. Plasma T_3 levels were elevated during early summer in both groups of fish. The percentages of surviving and smolted fish in seawater net-pens as of November of 1978 (0-age fish) and November of 1979 (yearling fish) are shown in Table 1. The percent survival, percent smolt, and percent surviving smolts of the groups entering seawater showed progressive increases during the time of the cycle of plasma T_4 in

Table 1. Comparison of seawater performance of groups of 0-age and yearling coho. Proportion of surviving and smolted fish was determined in November of the year of transfer.

Entry Group	Entry Date	% Survival (A)	% Smolt (B)	% Surviving Smolts (A x B)
		1978 Zero Age		
1	5/16	16	12	2
2	5/31	13	15	2
3	6/14	19	32	6
4	6/27	29	36	11
5	7/11	42	33	14
6	7/25	35	28	10
7	8/8	14	33	5
8	8/22	12	6	1
		1979 Yearling		
1	3/19	31	87	27
2	4/2	22	73	16
3	4/16	34	84	29
4	4/30	37	90	33
5	5/14	41	91	37
6	5/29	38	84	32
7	6/11	42	89	37
8	7/23	57	85	48
9	8/20	57	84	48

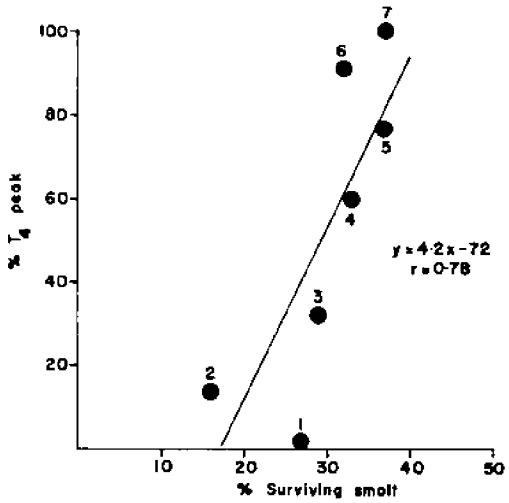

Figure 6. Relationship between percent T_4 peak and percent surviving smolt as of November 1979.

fish in freshwater (entry groups 2 through 5). An analysis of the percent of the T_4 peak (area under the curve) versus seawater performance of these groups of fish is shown in Figure 6. This relationship implies that fish entering seawater near the completion of the T_4 cycle in freshwater will show better long-term survival smolts of the 0-age fish transferred to seawater showed progressive increases through the first five entry groups (Table 1). This increase corresponds to the time of elevated plasma T_3 levels in fish in freshwater (Figure 5). A comparison of the proportion of the area under the T_3 peak at the time of seawater entry and percent surviving smolts is shown in Figure 7. This relationship implies that 0-age fish transferred to seawater near the end of the time of elevated plasma T_3 in freshwater show maximal seawater performance.

Analysis of plasma thyroid hormone concentrations in yearling chinook salmon (On-

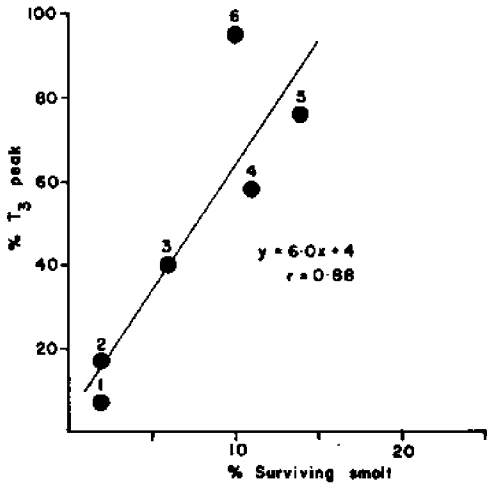

Figure 7. Relationship between percent T_3 peak and percent surviving smolt as of November 1979.

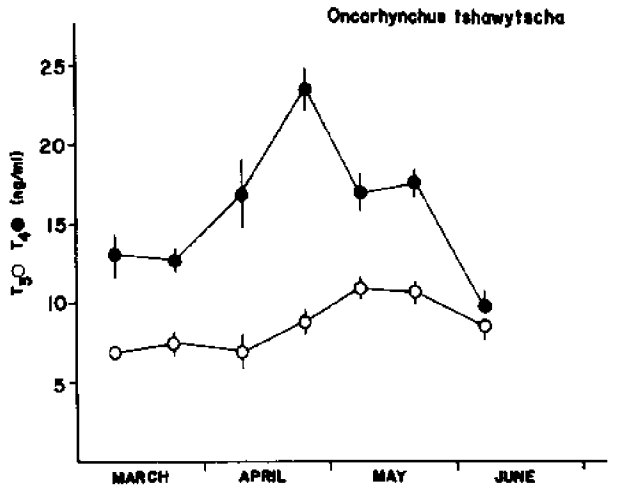

Figure 8. Pattern of change in plasma thyroid hormone concentration of chinook salmon during smoltification in freshwater.

corhynchus tshawytscha) and anadromous steelhead trout (*Salmo gairdneri*) also showed seasonal cycles during smoltification (Figure 8 and 9). This opens the possibility that such analyses may be useful for understanding the biology of smoltification of other salmonid fishes.

Discussion

The cycle of thyroid hormones during smoltification of coho salmon is a consistent, quantifiable phenomenon. Presumably, the elevated levels of T_4 and T_3 function to accelerate physiologic changes associated with the parr-smolt transformation. Premature transfer of fish from fresh- to seawater during the early stages of the thyroid hormone cycle causes a reduction in the plasma concentration of T_4. The depressed plasma T_4 of fish in seawater

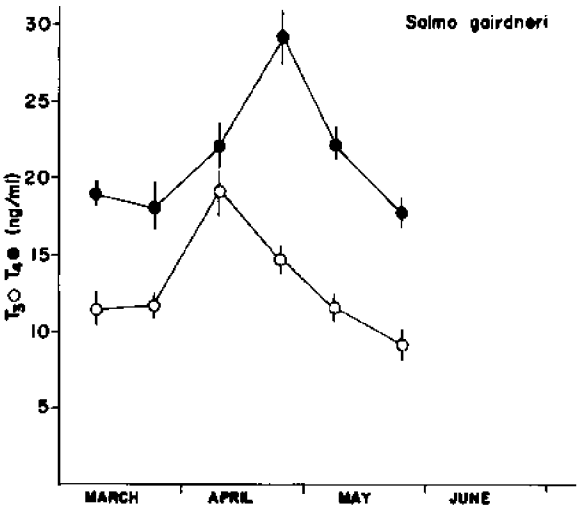

Figure 9. Pattern of change in plasma thyroid hormone concentration of steelhead trout during smoltification in freshwater.

may be due to lowered secretion of the hormone. Milne and Leatherland (1980) have recently presented evidence that the thyroid is less sensitive to thyroid stimulating hormone (TSH) in coho salmon in seawater. Furthermore, premature transfer of salmon to seawater causes stunting of growth associated with depressed thyroid activity (Clarke and Nagahama, 1977). Thus, the accumulated evidence suggests that the cycle of thyroid hormones in fish in freshwater plays an important role in determining the readiness of fish for seawater entry.

The cycles of T_4 and T_3 during the spring and early summer of both 0-age and yearling coho indicate that these events are seasonal occurrences happening every year regardless of the age or size of the fish. The relationship between seawater survival and time of entry in relation to the T_4 peak in yearling fish reared at the Northwest and Alaska Fisheries Center is similar to that observed for yearlings from the Columbia River hatcheries. The correlation of survival and the T_3 cycle of 0-age fish suggests that the physiological changes preparatory for seawater entry in this group occur by a mechanism which is different than that of yearlings. It may be that 0-age fish require a longer time of exposure to thyroid hormone for sufficient development for entry into seawater. Accordingly, their seawater performance would be optimal during early summer after they had experienced elevations of both plasma T_4 and T_3 concentration. The subsequent springtime elevation of T_4 in yearlings might then be sufficient for smoltification of the greatest proportion of the population. The seawater survival of 0-age fish in this study was generally poor. This is similar to the findings of Harache, et al. (1980). The poor performance may have been due to genetic factors since the stock used in this study (Toutle) had not been selected for 0-age maturation and were smaller than 0-age fish used in some aquaculture programs.

These results suggest that analysis of plasma thyroid hormone concentrations in salmon in freshwater may be a useful indication of the extent of smoltification and readiness for seawater entry. Additional confirmatory studies are presently underway. At this time these findings may be applicable only for net-pen culture operations in which fish are transferred directly to seawater. Many of the fish released from Columbia River hatcheries require 15 to 30 days to migrate to the estuary. We do not know how migration of fish may affect their plasma concentration of thyroid hormones or whether this might influence seawater performance.

Acknowledgment

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Sexual Maturation of Coho Salmon (*Oncorhynchus kisutch*): Accelerated Ovulation and Circulating Steroid Hormone and Ion Levels of Salmon in Freshwater and Seawater

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Summary

Coho salmon (*Oncorhynchus kisutch*) mature and spawn naturally in freshwater. We investigated the feasibility of artificially inducing ovulation of coho salmon retained in seawater during the spawning season and compared induced ovulation of coho salmon in seawater and in freshwater. Partly purified or purified salmon gonadotropin followed by luteinizing hormone-releasing hormone analogue, and whole chum salmon pituitary extracts were effective inducers of ovulation in salmon held in freshwater, and to a lesser extent in those held in seawater. Circulating levels of estradiol-17β, thyroxine, androgens, progesterone, sodium and osmolality were measured concomitant with determination of oocyte maturation in coho salmon held in freshwater and seawater during the spawning season. During the early stages of final gonadal maturation, sodium levels and osmolality of fish in seawater increased substantially, which suggested osmoregulatory difficulties. Also, there was a higher mortality rate of fish held in seawater. The circulating levels of all hormones measured differed between coho salmon in freshwater and those in seawater. We concluded that reproductive function is compromised in salmon retained in seawater due to osmoregulatory factors, which strongly influenced the maturational processes of these fish, and to high mortalities before ovulation.

Introduction

Reproductive control would be valuable in enhancing the propagation of Pacific salmon. Improper timing of spawning of the fish, reproductive failure, and prespawning mortality have been problems in hatcheries. Therefore, the development of techniques for accelerated spawning of salmon in hatcheries would be valuable.

Coho salmon, under normal circumstances, spend two years at sea and then enter freshwater streams from the ocean in the fall to spawn. Upon entrance into freshwater, the salmon undergo their final stages of sexual maturation. The final maturation stages of salmon are considered to consist of two distinct processes: maturation and ovulation. Jalabert (1976) defined oocyte maturation as, "those biochemical changes occurring at the completion of vitellogenesis." Ovulation is defined as "the release of the oocyte from the follicle." Not only do the salmon undergo their final maturation upon entrance into freshwater, but they experience many other morphological and physiological changes, some

of which may influence or interact with the maturational and ovulatory processes of salmon (Woodhead, 1975). Coho salmon that had returned to Weyerhaeuser's Ore Aqua facilities in Newport, Oregon, were retained in seawater during final maturation and ovulation and did not enter freshwater in this final phase. Those salmon that were held in seawater at the Ore Aqua facilities during the spawning season apparently developed osmoregulatory problems (Sower, 1980) and characteristically had high mortalities and low survival of the eggs. During 1977, 1978, and 1979 less than 35 percent, 68 percent and 40 percent, respectively, of the females were spawned; most of the fish died before ovulating. Thus, the biological problems faced by salmon retained in seawater during final maturation and ovulation are sizable. Consequently, one of the objectives of this study was to determine whether hormone administration to adult female coho salmon returning to seawater facilities enhanced maturation and ovulation, and to compare ovulation induced in salmon held in freshwater with that in fish held in seawater. To further aid in understanding the interrelationship between maturation and osmoregulatory processes of fish, serum sodium, osmolality and thyroxine were measured in fish in freshwater and seawater during the spawning season. Another objective was to describe the hormonal changes of coho salmon and relate their reproductive development in seawater to that in freshwater during final maturation and ovulation.

Materials and Methods

Hormone Induced Ovulation

On 28 September 1979, 70 female coho salmon (average weight 2.5 kg) were netted from seawater raceways at Newport, anesthetized (2 phenoxyethanol at 0.5 ml/l), tagged with Floy tags at the operculum (two tags per fish), weighed to the nearest 0.1 kg, and placed into a 4 m circular seawater tank. On 1 October, 70 more female coho salmon were netted and transported in seawater tanks from Newport to Weyerhaeuser's Jefferson facility, located one hour north of Corvallis, Oregon. They were anesthetized as above while still on the transport truck at Jefferson, tagged, and slid down a tube into a swimming pool 15 m long containing freshwater. The salmon held in seawater or freshwater were injected on 15 October and 16 October, respectively, as follows:

No. of fish		First Injection (mg/kg fish)	Second Injection (mg/kg fish)
SW	FW	Day 0	Day 3
9	7	Control (no injection)	Control (no injection)
10	8	Control (saline)	Control (saline)
10	11	SG-G100[1] (0.1)	LH-RH[2] (0.062)
12	10	GTH[3] (0.2)	LH-RH (0.062)
22	19	PIT[4] (10)	PIT (50)

[1]SG-G100, partly purified coho salmon gonadotropin.
[2]LH-RH, luteinizing hormone-releasing hormone analogue (D-Ala^6, des Gly^{10}—LH-RH ethyl amide).
[3]GTH, purified chum salmon gonadotropin.
[4]PIT, whole chum salmon pituitary preparation.

The fish from each of the freshwater and seawater treatment groups were checked twice weekly for maturity. At the time of ovulation, serum samples and a subsample of eggs were taken from each fish. Eggs were fertilized by at least two males, and reared to determine percentage survival.

The temperature and salinity of seawater at Newport in 1979 ranged between 9°C and 15°C

and 30 parts per thousand (ppt) and 32 ppt. The temperature of freshwater at Jefferson averaged 14°C.

Plasma Hormone Concentrations

Every two weeks from 14 September 1979 to 5 December 1979 ten female coho salmon, from fish held in either seawater or freshwater, were killed and sampled for serum and oocytes. These groups were considered the early entry groups. Comparative data were also collected from groups returning in the middle (26 September 1979) and late (15 October 1979) parts of their spawning run. The data from the middle and late entry groups were similar to results obtained in the early entry group and will not be reported here. Ten males were also sampled for serum during these sampling times. The fish in seawater were at Weyerhaeuser's Newport facility, while the fish in freshwater were at Weyerhaeuser's Jefferson facility.

The blood collected was held on ice for 30 minutes, centrifuged, the serum drawn off, and divided into two samples. Half of the serum was frozen at -20°C until analyzed for estradiol-17β, progesterone, androgens, and thyroxine. The other half of the serum was kept on ice and analyzed for sodium and osmolality the same day they were sampled. Samples of eggs from the fish were fixed in Stockard's fixing agent and analyzed immediately with an unaided eye or within a couple of days under a dissecting microscope. The gonadosomatic index was determined for each female salmon. Circulating estradiol-17β, progesterone, thyroxine, and androgens were determined by radioimmunoassay (Sower, 1980). Serum sodium and osmolality were determined by flame spectrophotometer and osmometer, respectively.

Statistics

Data for hormone concentrations were analyzed by a student Newman-Keuls test which was used for computing the significance of differences for samples of unequal sizes following preliminary analysis of variance. The accumulative percentage ovulation data were analyzed by a 2x2 contingency table followed by the Bonferonni approach (Neter and Wasserman, 1974). In all tests, the level of significance was $P < 0.05$).

Results

Hormone Induced Ovulation

No significant differences between the uninjected and saline injected controls were noted, so the data were combined and all controls were considered as one group. At day 17, significantly more experimental fish in freshwater than those in seawater had ovulated (Figure 1). In freshwater at day 17, ovulation had significantly occurred in 82 percent of the salmon treated with SG-G100 plus LH-RH, 50 percent of those treated with GTH plus LH-RH, and 42.2 percent of those treated with PIT compared with 0 percent in control fish. In seawater by day 17, percent ovulations were significantly higher in fish treated with SG-G100 plus LH-RH (90.9 percent) than in controls (40.0 percent).

By day 17, there was no mortality of fish held in freshwater and 47 percent mortality of fish held in seawater. The high mortality of fish in seawater occurred concurrently with high mortality among Ore Aqua's brood fish. Although the fish were screened for pathogens, no disease was detected in the fish held in seawater. Mortalities of fish held in freshwater, 32 days into the study, were due to an outbreak of furunculosis.

Plasma Hormone Concentrations

Peak spawning time for the salmon was during 5 and 6 December 1979, approximately three weeks later than the previous year. The GSI (percent) and egg diameter increased through final maturation with a few exceptions. The GSI and egg diameter of fish held in

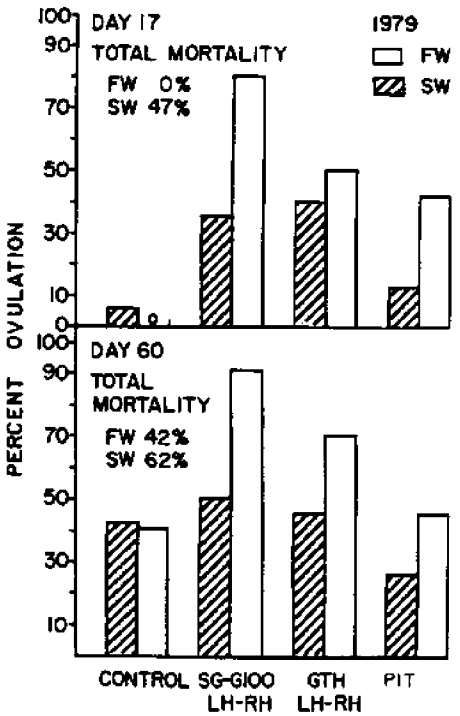

Figure 1. Accumulative percentage ovulations at days 17 and 60 of female coho salmon injected in 1979 with saline or uninjected (control), partly purified coho salmon gonadotropin (SG-G100) plus luteinizing hormone-releasing hormone analogue (LH-RH), or whole chum salmon pituitary (PIT). Open bars represent fish held in freshwater (FW) and hatched bars represent fish held in seawater (SW).

freshwater showed greater increases compared to the GSI and egg diameter of fish in seawater.

In most cases there was incomplete ovulation in fish in seawater. In some instances, half of the eggs in one skein (ovary) were overripe (semitransparent), while the eggs in the other ovary had not yet ovulated but appeared matured (germinal vesicle breakdown). Overripe eggs are those that are held in the body cavity for an extended period after ovulation.

Fish entered the seawater facility in Newport from the ocean on 17 September 1979. On 9 October 1979, both male and female coho salmon in seawater had significantly higher sodium levels than those of male and female fish in seawater on 2 October 1979 (Figures 2 and 3). Those values from 2 October 1979 are considered the normal values seen in salmon from seawater.

The sodium levels and osmolality for male salmon were slightly higher than the levels for female salmon held in either freshwater or seawater (Figures 2 and 3). Sodium levels and osmolality increased significantly in salmon held in seawater during the first part of October. Mortalities were beginning to increase at this time and remained high after this period. Osmolality and sodium levels varied little in female or male salmon held in freshwater.

The serum concentrations of estradiol-17β in female coho salmon showed an overall decrease from the time they entered the seawater facility to the time of ovulation regardless of whether they were held in freshwater or seawater (Figure 4). However, the levels of estradiol-17β tended to fluctuate during oocyte maturation. Estradiol-17β levels in female coho salmon in seawater during the early stages of oocyte maturation were significantly higher (6.38 ± 0.52 SE ng/ml) than levels in salmon in freshwater (3.96 ± 0.55 ng/ml) (Figure 4). At ovulation, estradiol-17β levels were significantly lower in the females which were held in seawater than those from fish in freshwater (Figure 4). Serum estradiol-17β levels in males were lower than in females and were not significantly different in either freshwater or seawater groups throughout the sampling period.

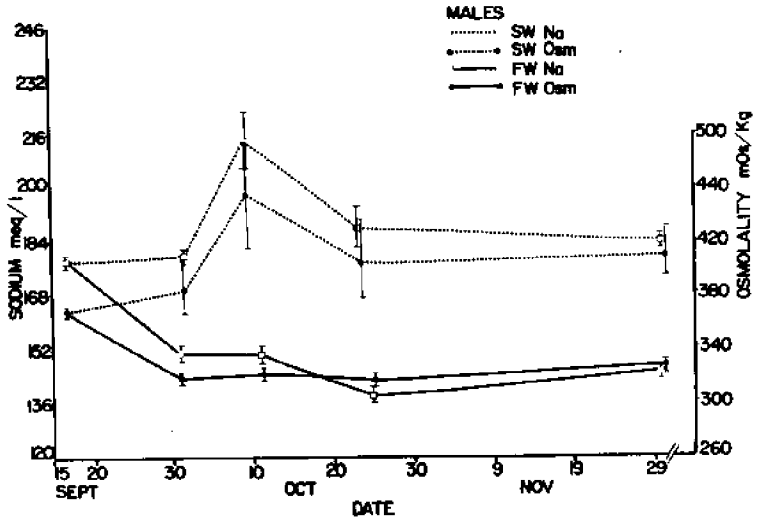

Figure 2. Mean serum sodium (Na)(meq/l) and osmolality (Osm)(mOs/kg) concentrations for male coho salmon held in seawater (SW) or freshwater (FW) during the final maturation of the spawning season from 15 September to 5 December 1979.

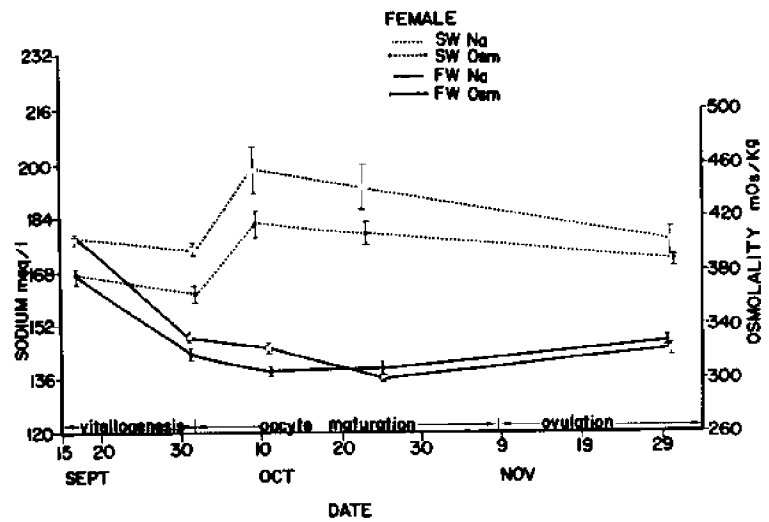

Figure 3. Mean serum sodium (Na)(meq/l) and osmolality (Osm)(mOs/kg) concentrations for female coho salmon held in seawater (SW) or freshwater (FW) during the final maturation of the spawning season from 15 September to 5 December 1979.

Progesterone levels of both males and females in freshwater were low and covaried with a range from 0.26 ± 0.03 to 0.72 ± 0.21 ng/ml (Figure 5). As with the estradiol-17β levels, progesterone levels during the early stages of final maturation in female and male salmon in seawater were elevated compared to those of salmon in freshwater. At spermiation and ovulation, progesterone levels were higher in male and female coho salmon in seawater compared to salmon in freshwater (Figure 5).

Thyroxine levels in serum of male salmon were higher than those in females in both seawater and freshwater (Figure 6). In the final stages of maturation, thyroxine levels were significantly higher in male salmon in freshwater compared to those found in female salmon

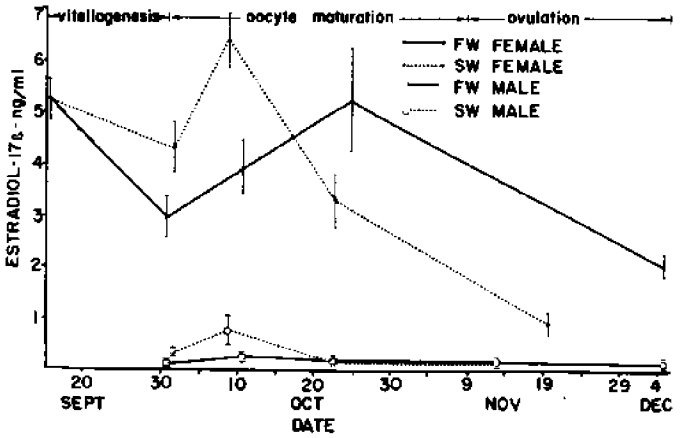

Figure 4. Mean serum estradiol-17β levels (ng/ml) for male and female coho salmon held in seawater (SW) or freshwater (FW) during the final maturation of the spawning season from 15 September to 5 December 1979.

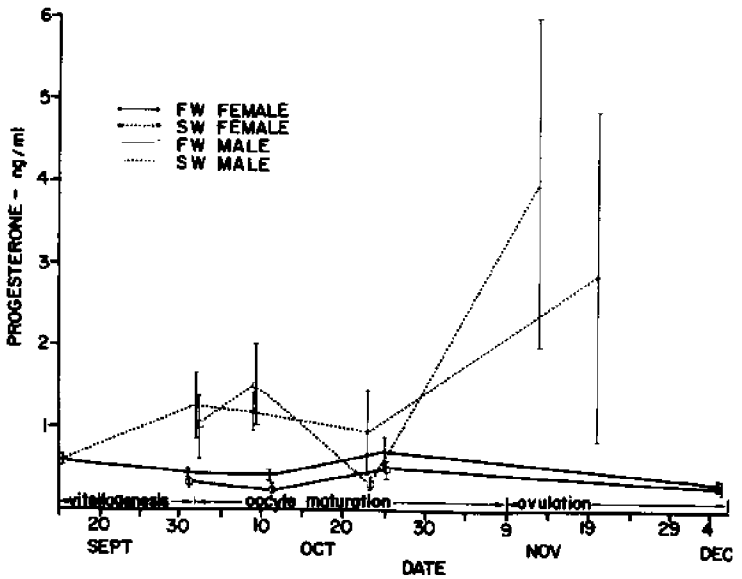

Figure 5. Mean serum progesterone (ng/ml) levels for male and female coho salmon held in seawater (SW) or freshwater (FW) during the final maturation of the spawning season from 15 September to 5 December 1979.

in freshwater (Figure 6). Serum thyroxine levels decreased throughout the spawning season in both male and female coho salmon in freshwater (Figure 6). At ovulation, thyroxine levels were higher in females in seawater compared to those of females in freshwater. Similarly, thyroxine levels were significantly higher in male coho salmon at spermiation in seawater than those of salmon in freshwater.

During the latter stages of final maturation, androgen levels in male salmon in freshwater were significantly higher than those of male salmon in seawater (Figure 7). Prior to ovulation and spermiation, androgen levels in both males and females in freshwater increased com-

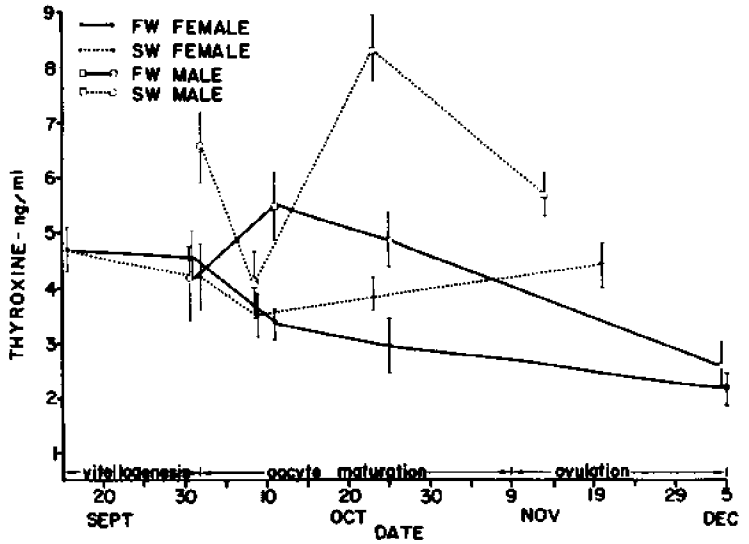

Figure 6. Mean serum thyroxine levels (ng/ml) for male and female coho salmon held in seawater (SW) or freshwater (FW) during the spawning season from 15 September to 5 December 1979.

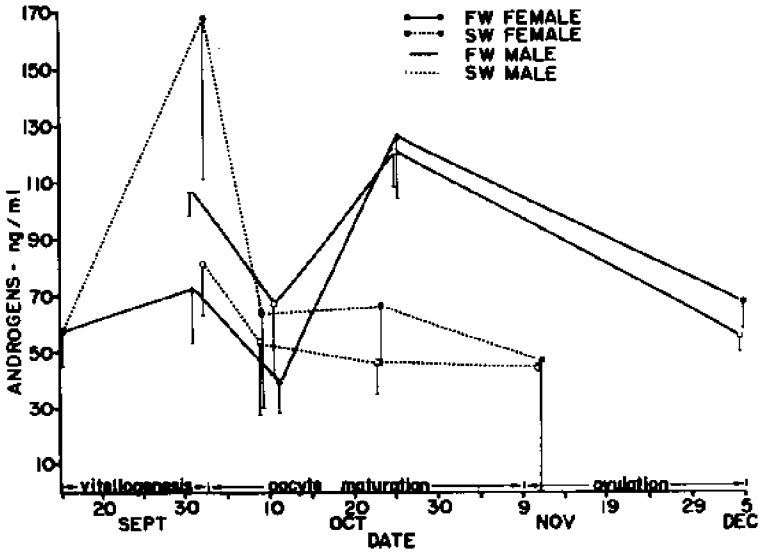

Figure 7. Mean serum androgen levels (ng/ml) for male and female coho salmon held in seawater (SW) or freshwater (FW) during the spawning season from 15 September to 5 December 1979.

pared to no elevation in those in males and females in seawater. Greater variation of androgen levels occurred in female compared to male salmon (Figure 7).

Discussion

Compounds that were effective inducers of ovulation in coho salmon in freshwater, and to a lesser extent in seawater, were SG-G100 plus LH-RH, GTH plus LH-RH, and PIT. Presumably, ovarian responsiveness to the administered hormones is mediated at some level of the hypothalamic-pituitary gonadal axis (Breton and Weil, 1973; Jalabert, 1976; Jalabert, et al., 1978; Peter, 1980). This response of the ovary to the hormones is dependent on the maturational stage of the female and on the dosage of hormone employed.

SG-G100 plus LH-RH, GTH plus LH-RH, and two injections of PIT induced ovulation at least six weeks earlier than the normal peak spawning time. It is evident that priming with gonadotropin or pituitary extracts is necessary for this ovulatory response, particularly when hormones are given more than six weeks from natural spawning. Donaldson, et al. (1979) demonstrated that treatment with LH-RH alone in coho salmon was less effective than treatment after the fish had been given a priming dose of chinook salmon SG-G100.

Hormonal treatments were less effective, or sometimes ineffective in inducing ovulation in salmon in seawater. Responses to treatment of the fish in seawater were reduced due to the high mortalities prior to ovulation, inhibiting factors associated with fish maturing in seawater or osmoregulatory difficulties. Normally, salmon undergo a change in osmotic environment when they enter freshwater from the ocean during final migration. We showed sodium and osmolality concentrations in fish in seawater had increased substantially during the early stages of final gonadal maturation. This is highly suggestive that osmoregulatory difficulties may have been the basis for the high adult mortality which was observed in fish held in seawater. There appears to be strong interrelationships of osmoregulatory and reproductive factors of female coho salmon remaining in seawater to spawn. For example, hormone profiles of estradiol-17β, thyroxine, androgens and progesterone differed between coho salmon in freshwater versus those in seawater. These different profiles of hormones of maturing salmon in seawater coincided with collapsed or dehydrated eggs, ovaries that were not completely ovulated and low egg survival. Some of these abnormalities may have been the result of incomplete oocyte maturation due to an ion imbalance in the egg (Sower, 1980). Therefore, osmoregulatory factors may inhibit or antagonize the reproductive process. The response of the ovary may also be desensitized by the hypersaline environment. Thus, if the returning salmon are prevented entry into freshwater as is normal, the endocrine system may not be able to response properly.

In summary, SG-G100 plus LH-RH, GTH plus LH-RH, and PIT in that order were effective for accelerating ovulation of coho salmon in freshwater and somewhat effective in salmon in seawater. Ovarian responses to the treatments of fish retained in seawater were reduced due to osmoregulatory difficulties or high adult mortality. It is apparent from the differences in hormone profiles, dehydrated eggs, small amounts of seminal fluid, incomplete ovulation, low egg survival and high adult mortality of salmon which are retained in seawater during the spawning season, that the reproductive function was compromised. Although functions of the various hormones in this study have not been clearly established, it is probable that some of them are active in reproduction. As stated earlier, the response of the ovary may be desensitized by the hypersaline environment which was indicated by generally higher circulating levels of hormones of salmon in seawater. Accelerated ovulation of coho salmon in seawater requires further research. However, in freshwater, hormones can be effective in enhancing early ovulation in coho salmon and thus overcome problems such as prespawning mortality.

Acknowledgements

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Section VII
Nutrition Requirements of Pacific Salmon

Proc. No. Pac. Aquaculture Symp.,
Aug. 1980, Anchorage, Alaska

Maturity Condition of Bristol Bay Sockeye Salmon [*Oncorhynchus nerka* (Walbaum)] in Summer in the Eastern Bering Sea

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Abstract

The interrelationships among seawater temperature, timing of spawning migration in the Kvichak River, growth rate and maturity condition in 2.2 age sockeye salmon were analyzed. The analyses were based on the data obtained from the central and southeastern Bering Sea from June through early July for an eight-year period from 1965 through 1972. An inverse correlation was found between the peak return date of sockeye salmon in the Kvichak River and the June mean water temperature in the study area. The peak return date was also related to the growth rate and maturity condition of the 2.2 age sockeye salmon in the ocean. In warm years, sockeye salmon with high growth rate, high maturity index, and low maturity increase rates returned earlier than those returning in cold water years. A positive correlation was obtained between the seawater temperature and the growth rate and maturity condition. It is believed that the seawater temperature regulates, simultaneously, the growth and maturation processes of sockeye salmon in the ocean prior to ascending migration, and thus determines the timing of ascending migration. The somatic and gonadal development of sockeye salmon seems to be accelerated by warm sea temperatures, causing sockeye salmon to ascend earlier than they do in cold water years.

Introduction

Bristol Bay sockeye salmon returned to their natal rivers during the period from the end of June through July, with a peak in the first two weeks of July (Royce, et al., 1968). The duration of spawning migration and the date of peak return fluctuated from year to year. Nishiyama (1977) has stated that the peak return date of sockeye salmon in the Kivchak River is related to the June seawater temperature in the central and southeastern Bering Sea: the arrival of sockeye salmon at the mouth of the river began earlier in the warm water temperature than in the cold water temperature. Burgner (1978) has shown that the date of the 50 percent points of annual run for Bristol Bay sockeye salmon is related to the air temperature and monthly sea surface temperature deviations south of the Aleutian area between the Alaska Peninsula and Adak Island. These findings suggest that the timing of spawning migration of sockeye salmon is dominated by oceanographic and climatic conditions. Although the timing of spawning migration seems to have resulted from direct and indirect influence of water temperature upon the biological process of sockeye salmon in the ocean, the mechanisms involved remain uncertain.

In this paper, analyses are attempted to examine the interrelationships among seawater temperature, timing of spawning migration, growth rate and maturity condition of sockeye salmon, focusing on the 2.2 (5_3) age fish and their peak return date in the Kvichak River.

Data Sources

In this report the data relating to water temperature, period of spawning migration, growth rate and maturity condition of sockeye salmon were obtained from existing materials.

Water Temperature

The mean sea surface water temperatures in the central and southeastern Bering Sea area (north of 60°N and east of 180°), inhabited by Bristol Bay sockeye salmon from early June through early July, have been reported for the eight years between 1965 and 1972 (Nishiyama, 1977). In this study, the rate of temperature increase per each ten-day period in June has been calculated.

Peak Return Date

The period of spawning migration, daily catch, and escapement data of sockeye salmon in the Kvichak River from 1965 through 1972 have been compiled by Pennoyer and Seibel (1966), Pennoyer (1967), McCurdy and Pennoyer (1968), McCurdy and Paulus (1972), Paulus and Nelson (1972a and b), McCurdy and Schroeder (1972), and Krasnowski and Randall (1975). The date the maximum return was counted is referred to as the peak return date. The peak return date is assumed to represent the timing of spawning migration of a given year.

Growth Rate and Maturity Condition

The daily growth and gonadal development rates for the 2.2 age sockeye salmon population in the central and southwestern Bering Sea in June and early July have been given by the author (1975 and 1977). The mean body (somatic) weight, mean gonad weight, and maturity index (the mean percent of gonad weight to body weight) have been recorded. The rate of increase of the maturity index per ten-day period in mid-June to early July is calculated based on these data.

Results

Water Temperature Condition

The mean seawater surface temperature in the central and southeastern Bering Sea is given for each ten-day period of June and early July (Table 1). Throughout the eight years the mean water temperature varied between 3.95° and 8.49°C, showing a tendency to increase as the season advanced. The average mean water temperature in June is 5.4°C. Based on the criteria of the average water temperature, the years of 1965, 1971 and 1972 were regarded as cold water years, whereas the years of 1967 and 1969 were warm water years. The warmest temperatures were recorded in 1967 while the coldest occurred in 1972.

Table 1. Surface mean water temperature in the central and southeastern Bering Sea. The figures in parentheses indicate the number of measurements. (Date from Nishiyama, 1977.)

	June				July
Year	Early	Middle	Late	Mean	Early
1965	4.62(22)	5.18(25)	4.62(28)	4.81	—
1966	5.16(29)	5.40(53)	6.20(11)	5.59	—
1967	5.68(33)	6.05(56)	8.49(18)	6.74	—
1968	5.09(8)	5.38(42)	6.25(53)	5.57	—
1969	5.27(20)	5.87(77)	6.89(76)	6.01	8.36(12)
1970	5.14(67)	5.78(87)	6.06(87)	5.66	6.55(52)
1971	4.58(53)	4.41(53)	4.78(76)	4.59	5.23(67)
1972	4.10(44)	3.95(77)	4.72(76)	4.26	5.36(49)

Figure 1 presents the relationship between the mean water temperature of June and the rate of temperature increase per each ten-day period in June. The rate of temperature increase varied considerably by year, but was found to be positively correlated to the mean water temperature of June. The relationships are expressed by a straight line and the regression is statistically significant at the 95 percent level ($F = 24.2$, $0.025 < P < 0.05$). This indicates that the water temperature rose faster in the warm water years than in the cold water years.

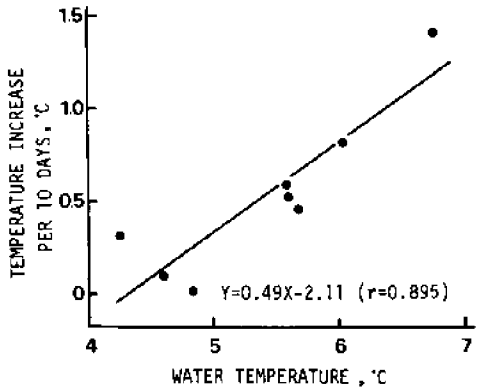

Figure 1. Relation between mean surface water temperature and rate of temperature increase per ten-day period in June, 1965 to 1972.

Peak Return Date of Sockeye Salmon in the Kvichak River

The daily return of sockeye salmon to the Kvichak River is illustrated in Figure 2. The beginning and the end of migration fluctuated from year to year. The earliest return occurred on 20 June in 1965 and 1967, and the latest was recorded on 1 July in 1971. The return ended on 24 July in 1972, while it continued until 7 August in 1970.

The annual fluctuation of the peak return date is obvious. The peak return was observed on 1 July in 1967 and on 11 July in 1971, with a difference of ten days. There is no apparent relationship between the peak return date and the total escapement.

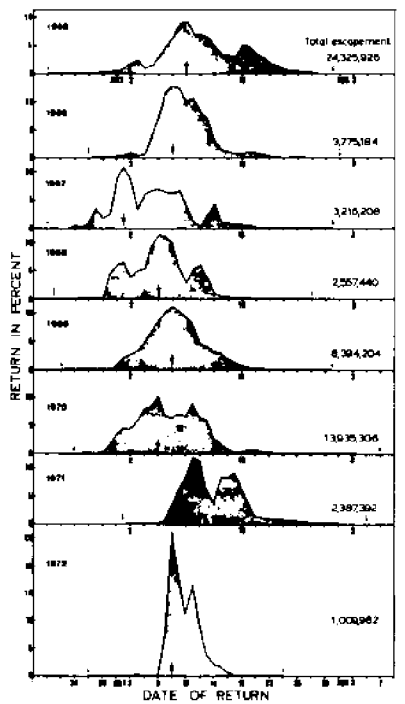

Figure 2. Duration of return of sockeye salmon to the Kvichak River. The daily return is given in percentage frequency. Small arrows indicate the date of beginning (left) and the end (right) of ascending migration. Large arrows indicate the peak return date.

Growth Rate and Maturity Condition

Table 2 presents the mean body weight and gonad weight of the 2.2 age sockeye salmon taken from the study area. The mean body weight varied from 1,817 to 2,157 g in females and from 2,060 to 2,511 g in males. Figure 3 shows that the mean body weight is inversely related to the total 2.2 age population size. The relationships can be fitted to exponential curves with a high statistical significance at the 95 percent level (female $F = 22.5$, $0.025 < P < 0.05$: male $F = 12.6$, $0.01 < P < 0.025$).

The mean gonad weight ranged between 73 and 128 g in females and 32 and 83 g in males (Table 2). The maturity index ranged from 2.12 to 5.81 in females and from 1.11 to 3.10 in males (Table 3). No apparent relationship was found between the total 2.2 age population size and mean gonad weight and maturity index.

Table 4 represents the growth rate and gonadal development rate. It is evident that annual

Table 2. Mean body weight and mean gonad weight of 2.2 age sockeye salmon in the central and southeastern Bering Sea in June. The number of samples is given in parentheses. (Data from Nishiyama, 1975 and 1977.)

	Body weight (g)		Gonad weight (g)	
Year	Female	Male	Female	Male
1965	1,817(79)	2,060(180)	90(79)	49(119)
1966	2,054(67)	2,329(25)	83(105)	60(51)
1967	2,171(524)	2,450(446)	92(530)	32(436)
1968	2,157(230)	2,459(171)	79(229)	68(188)
1969	2,066(439)	2,313(402)	128(470)	83(401)
1970	1,977(485)	2,264(634)	84(498)	50(646)
1971	2,033(315)	2,269(317)	101(315)	46(312)
1972	2,141(368)	2,511(337)	73(368)	33(335)

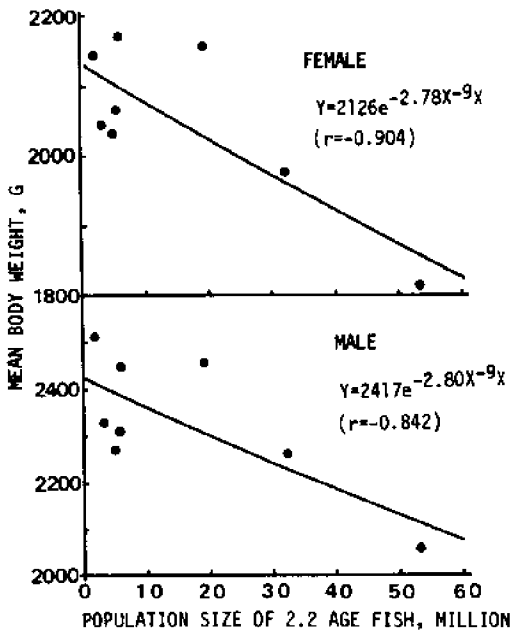

Figure 3. Relation between population size and mean body weight in 2.2 age sockeye salmon.

Table 3. Maturity index of 2.2 age sockeye salmon in the central and southeastern Bering Sea. (Data from Nishiyama, 1975.)

	Female			Male		
Year	Mid-June	Late June	Early July	Mid-June	Late June	Early July
1965	4.52	5.50	—	2.08	2.83	—
1966	3.67	4.69	5.03	—	2.32	—
1967	4.01	4.47	4.15	1.66	2.21	2.32
1968	3.51	4.08	4.69	1.84	2.09	1.94
1969	4.58	5.81	5.52	2.52	3.10	2.76
1970	4.31	3.94	4.58	2.20	2.13	2.56
1971	2.12	3.96	4.13	1.48	1.81	2.10
1972	3.25	3.80	4.25	1.11	1.74	1.84

Table 4. Daily growth rate and gonadal development rate of 2.2 age sockeye salmon in the central and southeastern Bering Sea in June. (Data from Nishiyama, 1977.)

	Growth rate		Gonadal development rate	
Year[1]	Female	Male	Female	Male
1965	0.520	0.062	1.31	3.89
1967	1.008	1.024	1.40	2.71
1968	0.189	0.665	1.89	0.82
1969	0.190	0.239	-0.74	-2.02
1970	0.397	0.425	0.16	0.86
1971	0.407	0.518	-1.99	0.05
1972	-0.091	0.046	1.52	3.11

[1]The data in 1966 were unavailable due to insufficient sample size.

fluctuation occurred in both growth and gonadal development rates. The reasons for the negative values have not yet been examined. There are no apparent relationships between the total 2.2 age population size and growth rate, though the growth rate tended to decrease with an increase in population size (Figure 4). Likewise, a relationship was not found between the total 2.2 age population size and the gonadal development rate.

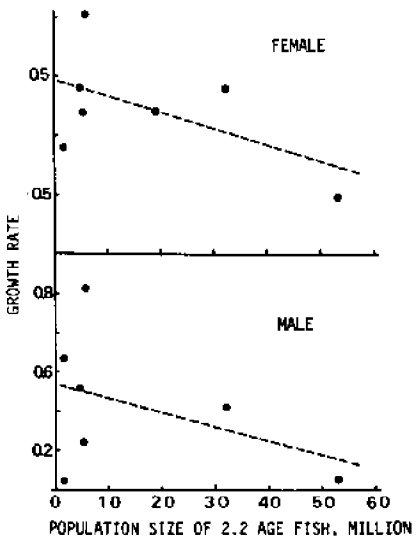

Figure 4. Relation between population size and growth rate in 2.2 age sockeye salmon.

It is evident that the gonadal development rate is negatively related to the mean gonad weight (Figure 5). The relation shows that the smaller the mean gonad weight, the greater the gonadal development rate. Logarithmic regression is well fitted to the set of these data, and is significant at the 90 percent level (female $F = 4.5$, $0.05 < P < 0.1$: male $F = 6.0$, $0.05 < P < 0.1$). This may suggest that the maturation processes are active in the small gonads. No apparent relationship was found between the gonadal development rate and the daily growth rate.

These results indicate that the mean fish size is dependent on the population size, but the growth and gonadal development rates are irrespective of the population size. The gonadal development rate seems to be a function of the gonad weight.

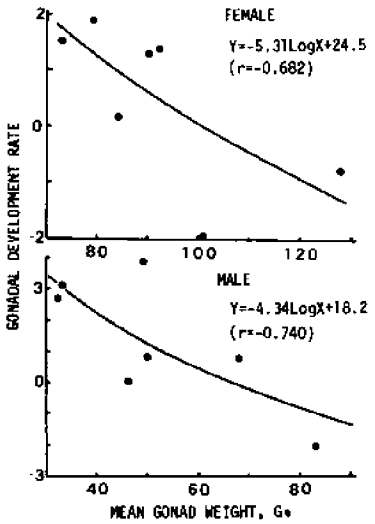

Figure 5. Relation between mean gonad weight and gonadal development rate in 2.2 age sockeye salmon.

Relation of Peak Return Date to Water Temperature, Growth Rate, and Maturity Condition

The peak return date of sockeye salmon to the Kvichak River is plotted against the mean water temperatures (Figure 6). Obviously, there are inverse relationships between the peak return date and the water temperatures. The relationships are expressed by negative linear regression lines. The regression is highly significant at the 95 percent level for early June ($F = 6.1$, $0.025 < P < 0.05$), late June ($F = 18.3$, $0.005 < P < 0.01$) and mean June temperature ($F = 10.4$, $0.01 < P < 0.025$), while it is not significant at the 90 percent level for mid-June ($F = 3.6$, $0.1 < P < 0.25$). Figure 7 demonstrates the relationship between the peak return date and the rate of temperature increase per each ten-day period in June. This regression is highly significant at the 95 percent level ($F = 24.5$, $0.025 < P < 0.05$). These results indicate that sockeye salmon returned earlier during the warm water temperatures of June with a higher temperature increase rate than in cold water temperatures.

Figure 8 shows that there are inverse relationships between the growth rate and the peak return date. Apparently, the higher the growth rate, the earlier the return occurred. The linear regression curves are well fitted to these data. The regression is statistically significant at the 90 percent level (female $F = 5.4$, $0.05 < P < 0.1$; male $F = 6.1$, $0.05 < P < 0.1$). This result suggests that the peak return date depends on the growth rate of sockeye salmon in the ocean.

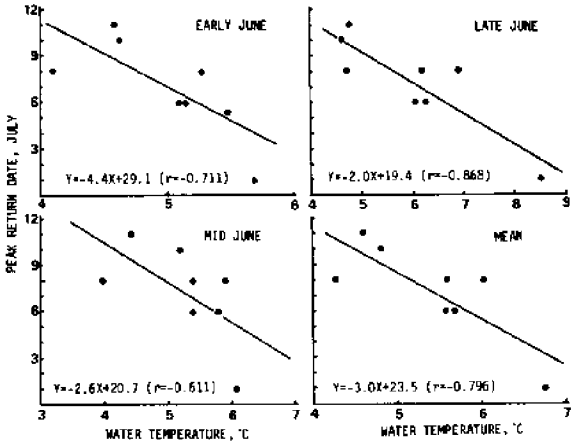

Figure 6. Relation between water temperature of June and peak return date of sockeye salmon in Kvichak River.

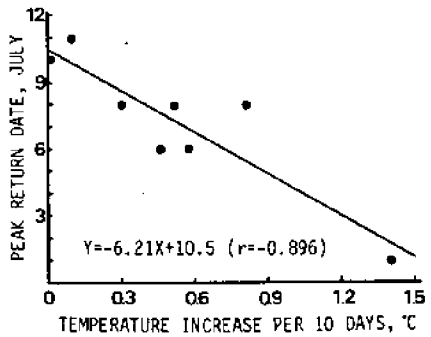

Figure 7. Relation between the rate of temperature increase per ten-day period in June and peak return date of sockeye salmon in the Kvichak River.

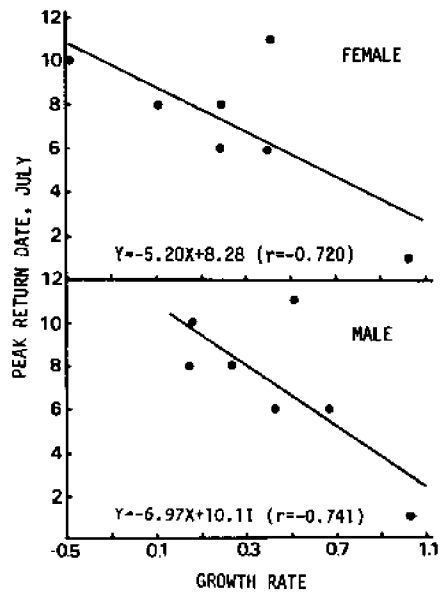

Figure 8. Relation between growth rate of 2.2 age sockeye salmon in June and peak return date of sockeye salmon in the Kvichak River.

The peak return date exhibited a close relation to the rate of maturity index increase per each ten-day period in June (Figure 9). The peak return date occurred earlier in the years with a high increase rate of maturity index. The linear regression lines are fitted to these relations, and the regression is statistically significant at the 95 percent level for females (F = 12.9, $0.01 < P < 0.025$) and at the 90 percent level for males (F = 4.5, $0.1 < P < 0.25$). The results imply that the maturity condition is associated with the timing of spawning migration of sockeye salmon.

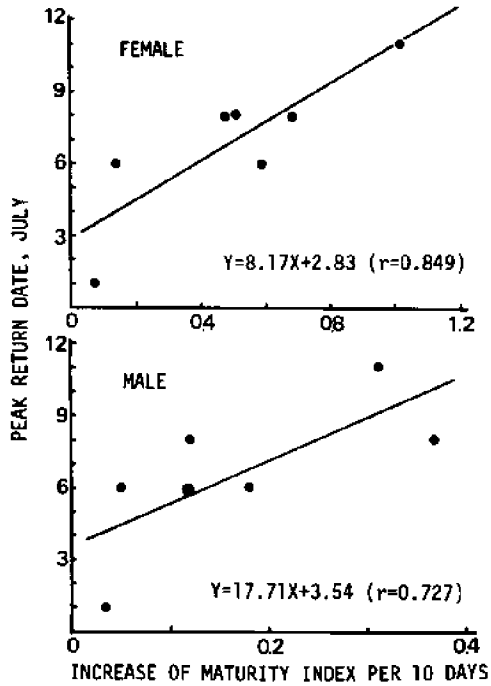

Figure 9. Relation between rate of increase of maturity index per ten-day period in middle June to early July in 2.2 age sockeye salmon and peak return date of sockeye salmon in the Kvichak River.

Relation of Water Temperature to Growth Rate and Maturity Condition

The relationships between the mean water temperature of June and the daily growth rate of sockeye salmon are shown in Figure 10. The growth rate increased steadily with an increase in water temperature. The linear regression is statistically significant at the 90 percent level (females F = 4.7, $0.05 < P < 0.1$; males F = 5.1, $0.05 < P < 0.1$). Similarly, the growth rate is found to be related to the rate of temperature increase per ten-day period in June (Figure 11). The regression is highly significant at the 95 percent level in females (F = 7.6, $0.025 < P < 0.05$) and at the 90 percent level in males (F = 5.3, $0.05 < P < 0.1$). These results indicate that the growth rate of sockeye salmon in the ocean is regulated by the water temperature and the rate of temperature increases in June. It appears that in the warm water years, the growth of sockeye salmon is more accelerated than in the cold water years.

There are positive relationships between the water temperature of mid-June and the maturity index (Figure 12). The linear regression is significant at the 90 percent level in females (F = 3.8, $0.05 < P < 0.1$) and the 95 percent level in males (F = 6.61, $0.025 < P < 0.05$). The mean water temperature of June appears to be inversely related to the rate of increase of the maturity index (Figure 13). The linear regression is statistically significant at the 95 percent level in males (F = 20.0, $0.01 < P < 0.025$), but is not significant at the 90 percent level in females; (F = 3.7, $0.1 < P < 0.25$). These relationships indicate that the rate of increase of the maturity index is greater in the cold water years. There is a smaller maturity index in the warm water years.

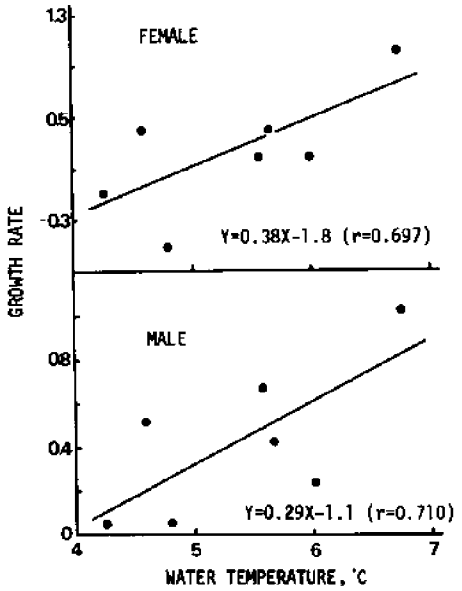

Figure 10. Relation between mean water temperature of June and growth rate of 2.2 age sockeye salmon.

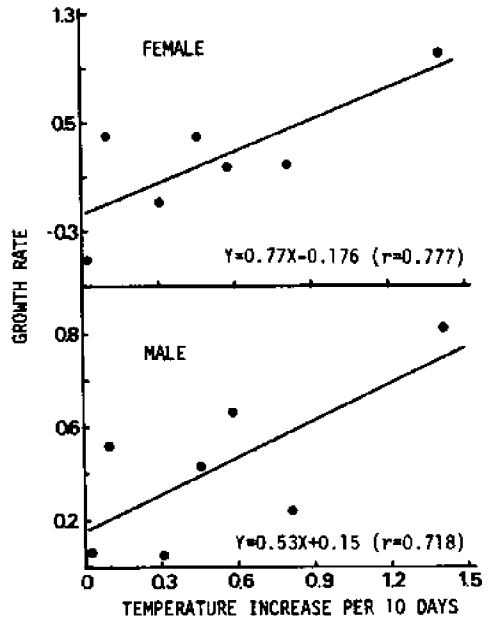

Figure 11. Relation between rate of temperature increase per ten-day period in June and growth rate of 2.2 age sockeye salmon in June.

Conclusion

The results of the present analyses show that there exist interrelationships among water temperature, timing of spawning migration, growth rate, and maturity condition of sockeye salmon. The peak return date exhibits an inverse correlation to the seawater temperature and the increased rate of temperature in June. It is certain that sockeye salmon returned to the river earlier in the warm water years than in the cold water years. The results have also revealed that the growth rate and maturity condition of sockeye salmon in the ocean are closely related to the peak return date. Sockeye salmon ascended earlier in the years with

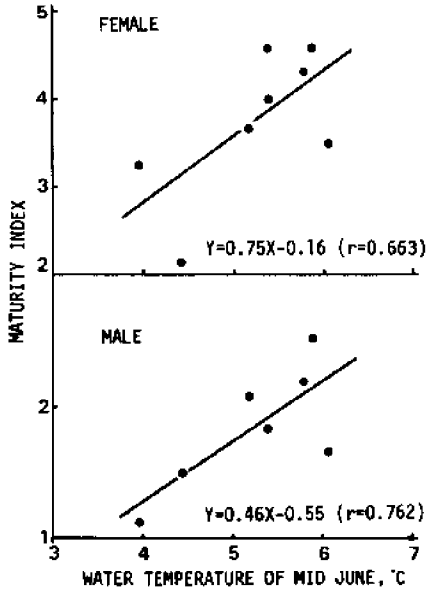

Figure 12. Relation between mean water temperature of middle June and maturity index of 2.2 age sockeye salmon in June.

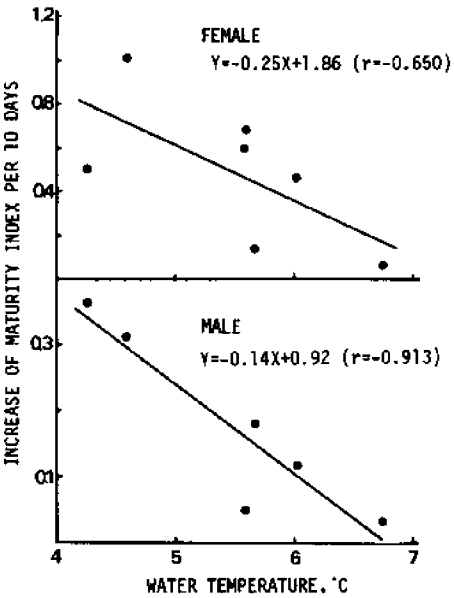

Figure 13. Relation between mean water temperature of June and rate of increase of maturity index per ten-day period in middle June to early July.

high growth rate and high maturity condition than in the years with low growth rate and low maturity condition. This phenomenon leads to the assumption that the seawater temperature is involved in determining the timing of spawning migration, as a result of its influence upon growth and maturation. The analyses show that the seawater temperature of June is positively correlated to growth rate and maturity condition. The growth rate and maturity condition were higher in the warm water years than in the cold water years. This suggests that the seawater temperature of June regulates the growth rate and maturity condition of sockeye salmon in the ocean prior to ascending migration. There should be a causal sequence between water temperature and biological process of sockeye salmon. It can be simply for-

mulated that in the first place the seawater temperature regulates the growth rate, then the growth rate affects the maturity condition, and finally, the maturity condition determines the timing of spawning migration. However, since there was no strong evidence to demonstrate the correlation between growth rate and maturity condition in the present analyses, this simple formulation will not be adequate. Alternatively, it appears that the water temperature regulates simultaneously the growth and maturation in different ways, and finally the influence of water temperature upon the growth and maturation results in the determination of ascending migration. The influence of growth rate upon maturation process awaits further analysis in a future study.

It should be noted that when the seawater temperature was higher, sockeye salmon returned earlier, accompanied by a higher growth rate and a higher maturity index, but a lower maturity increase rate. This suggests that sockeye salmon could have advanced growth and gonadal development in the ocean more rapidly in the warm water years than in the cold water years, instead of a shorter stay in the ocean. This may be ascertained by the positive correlation between water temperature and maturity index and growth rate. The difference in growth rate and maturity condition between warm water years and cold water years may suggest that there is a difference in food availability caused by water temperature condition. It is assumed that in the cold water years food availability was lower than in the warm water years. Thus, sockeye salmon must remain longer in the ocean until necessary organic matter is obtained for somatic and gonad production.

Fisheries management and industry in the Kvichak area will benefit from information which can forecast the peak return date of sockeye salmon. The peak return date of sockeye salmon in the Kvichak River (Z) will be given by the following multiple linear regressions based on the mean water temperature of June (T), the growth rate (GR) or the maturity index (MI) of the 2.2 age fish in June:

Female $Z = 19.88 - 2.28T - 2.08GR$ $(r = -0.841)$
Male $Z = 20.03 - 2.17T - 2.98GR$ $(r = -0.848)$

or

Female $Z = 13.93 - 3.47T + 2.67MI$ $(r = -0.970)$
Male $Z = 20.31 - 4.18T + 4.39MI$ $(r = -0.975)$

These equations indicate that the data on the seawater temperature, growth and maturity condition are useful to predict the peak return date. The accuracy should be improved by obtaining these kinds of data systematically in the central and southeastern Bering Sea.

Although the relation of water temperature to the maturity condition was analyzed, the temperature effect upon fecundity of sockeye salmon has not been studied in this paper. The variation of temperature and maturity condition suggests a positive relation between sea temperature and fecundity. This must be analyzed in the future.

Acknowledgements

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Essential Fatty Acids and Nutritive Value of Dietary Lipids for Marine Fish

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A series of studies on the nutritive value of lipids for marine fishes clarified that marine fishes have a considerably different requirement for essential fatty acids than terrestrial animals and freshwater fishes. In this paper, therefore, an outline of these studies is presented in accordance with the historical progress.

Nutritive Value of Dietary Lipids

It is well known that arachidonic acid (20:4 ω6) plays an important role in the nutritional physiology of rats and poultry. Since arachidonic acid can also be converted from linoleic acid *in vivo*, vegetable oils such as soybean and corn oil which are rich in linoleic acid are rated high nutritionally at the present time. However, wild marine fishes grow normally by feeding on marine organisms which are poor in the ω6 series fatty acids and linolenic acid (18:3 ω3), but are rich in ω3 highly unsaturated fatty acids with more than 20 carbon atoms (HUFA). Therefore, we presumed that the requirement of marine fishes for essential fatty acids may be different from that of terrestrial animals, and that the nutritive value of vegetable oils may be inferior to that of fish oils. From this standpoint, the nutritive value of vegetable oils and fish oils for marine fishes, such as red sea bream (*Chrysophrys major*) and yellowtail (*Seriola quinqueradiata*), was compared using a compounded diet containing pollock meal. Consequently, some workers demonstrated that a diet containing corn oil as a sole lipid source did not support as rapid a growth rate as a diet containing fish oil (Yone, 1966; Tsukahara, et al., 1967; Yone, et al., 1971). In the subsequent studies with a purified type diet (Table 1), Yone and co-workers obtained the same results. Namely the growth and feed efficiency of red sea bream (Yone and Fujii, 1975) and yellowtail (Yone, 1978) receiving corn oil were noticeably inferior to those of the fish receiving pollock liver oil, as shown in Table 2. Soybean oil also showed low nutritive value for red sea bream (Table 3) (Yone, et al., 1971). Recently, Cowey, et al. (1976a), reported that turbot (*Scophthalmus maximus*) fed on the diets with corn oil and hardened palm oil at a 4 percent level for 16 weeks showed lower growth and feed efficiency than with cod liver oil.

Based on these results, it can be concluded that vegetable oils rich in linoleic acid are not suitable as a dietary lipid source for marine fishes.

Essential Fatty Acid

Lee, et al. (1967) showed that corn oil in a purified diet led to poor growth and high mortality of rainbow trout (*Salmo gairdneri*), and also that the substitution of salmon oil or linolenic

Table 1. Composition of basal diets.

Diets	YR-1	YR-5	YR-6	Y-10	YY-2	Minerals	*2	*3	*4
Casein	52	54	54	57	64.2	$NaH_2PO_4 \cdot 2H_2O$	30.805	62	—
Gelatin	11	12	12	12	13.5	KH_2PO_4	—	—	15
L-Phe	0.6	0.6	0.6	—	—	Ca-lactate	19.74	—	15
L-Arg•HCl	1.3	1.5	1.5	—	—	$CaH_4(PO_4)_2 \cdot H_2O$	—	—	33
L-Cys	0.7	—	—	0.3	0.345	KCl	5.18	10	—
L-Try	0.2	0.2	0.2	—	—	$MgSO_4 \cdot 7H_2O$	—	—	10
L-His•HCl•H_2O	0.2	—	—	1.5	1.725	Fe-citrate	1.485	6	7
L-Lys•HCl	0.6	—	—	0.2	0.23	$AlCl_3 \cdot 6H_2O$	0.009	0.018	0.015
L-Val	0.7	0.7	0.7	—	—	$ZnSO_4 \cdot 7H_2O$	0.1785	0.357	0.300
Pollock liver oil	9	9	9	9	9	$MnSO_4 \cdot 4\text{-}6H_2O$	0.04	0.08	0.08
Dextrin	8	8	8*1	9	0	CuCl	0.0055	0.011	0.01
Minerals	8*2	5*3	6*5	7*4	7*4	KI	0.0085	0.017	0.015
Vitamins*5 +α-cellulose	5	8	7	3	3	$CoCl_2 \cdot 6H_2O$	0.0525	0.105	0.100
Attractants	2.7*6	1*7	1*7	1*8	1*6	Dextrin	—	—	15
						α-Cellulose	43.084	21.412	4.48

*1α-Starch, *2Halver (1957), *6DL-Ala, 1.3; L-Asp•Na, 1.0; Gly, 0.4, *7L-Asp•Na, *8DL-Ala, 0.3; L-Asp•Na, 0.3; L-Glu•Na, 0.368; 5'-Ribonucleotide•Na, 0.032.

Table 2. Effects of corn oil and pollock liver oil on the growth and feed efficiency of red sea bream and yellowtail.

	Red sea bream*1		Yellowtail*2	
Fishes Dietary Lipids	Pollock	Corn	Pollock	Corn
No. of fish				
at start	23	23	30	30
dead	0	0	4	11
Ave. body wt. (g)				
at start	22.6±1.1*3	22.6±1.1	13.7± 1.5	13.6±1.5
at end	43.8±7.8	29.3±4.1	52.6±15.2	17.0±3.3
"t-test"	—	S*4	—	S
Gain (g)	21.2	6.7	38.9	3.4
Feed efficiency (%)	57.4	23.7	98.9	12.7

Diet: *1 YR-5, *2 Y-10. Feeding period: *1 45 days, *2 28 days, *3 Standard deviation, *4 Significant at a 5% level.

Table 3. Effects of soybean oil and pollock liver oil on the growth and feed efficiency of red sea bream.

Dietary Lipids	Pollock*1	Soybean
No. of fish		
at start	20	20
dead	3	3
Ave. body wt. (g)		
at start	41.2±4.5*2	40.9±4.2
after 28 days	50.9±6.2	46.0±4.7
"t-test"	—	S*3
Gain (g)	9.7	5.1
Feed efficiency (%)	59.9	39.8

*1 YR-1, *2 Standard deviation, *3 Significant at a 5% level.

acid for a part of the dietary corn oil improved its nutritive value. Subsequently, Castell, et al. (1972a), concluded that linolenic acid has an essential role in the nutrition of rainbow trout similar to that assigned to linoleic acid in terrestrial animals. Furthermore, Watanabe, et al. (1975), in carp (*Cyprinus curpio*) and Takeuchi, et al. (1980), in eel (*Anguilla japonica*) demonstrated that linolenic acid is essential in nutrition. On the other hand, pollock liver oil scarcely contains linoleic and linolenic acid, whereas it showed remarkably higher nutritive

value than soybean oil rich in linoleic and linolenic acid for marine fishes (Table 4). From these findings, Yone and Fujii (1975) presumed that the essential fatty acids of marine fishes may be different from those of freshwater fishes, and examined the effect of supplementing linolenic acid to the diet containing corn oil on the growth and feed efficiency of red sea bream. As shown in Table 5, the growth and feed efficiency of the fish fed linolenic acid were as poor even at a 4 percent level as those of the corn oil diet group and the fat-free diet group, and were remarkably inferior to those of the pollock liver oil diet group (Fujii and Yone, 1976). In the study using laurate as a main lipid source instead of corn oil (Fujii and Yone, 1976), the group fed laurate only showed significantly lower growth and feed efficiency than those of the fat-free diet group (Table 6). The linolenic acid supplement elevated the nutritive value of laurate with the increasing of the dietary level. However, the growth rate

Table 4. Fatty acid composition of pollock liver oil, corn oil, soybean oil, and tallow.

	Pollock	Corn	Soybean	Tallow
14:0	5.9	tr	tr	3.1
15:0	0.5			0.9
16:0	11.5	10.5	11.4	25.9
16:1 ω7	7.6	0.4		4.7
18:0	2.1	2.1	4.2	18.4
18:1 ω9	13.3	28.6	23.0	37.2
18:2 ω6	1.2	55.7	54.2	3.0
18:3 ω6	0.5	1.4		1.3
18:3 ω3	tr	0.8	7.5	tr
20:1 ω9	20.1			
20:2 ω9	0.5			
20:3 ω9	tr			
20:3 ω6	0.5			
20:4 ω6	tr			
22:1	15.6			
20:5 ω3	10.9			
22:4 ω6	0.6			
22:5 ω6	tr			
22:5 ω3	1.3			
22:6 ω3	4.3			

Table 5. Effect of linolenic acid (18:3 ω3) supplement in corn oil diet on growth and feed efficiency of red sea bream.

Dietary lipid 18:3	0	0	0.5	1	2	4	Pollock
(%) corn	0	9	8.5	8	7	5	(YR-1)
No. of fish							
at start	25	25	25	25	25	25	25
dead	0	0	0	0	0	1	0
Ave. body wt. (g)							
at start	20.9 ±1.3	21.0 ±1.2	21.0 ±1.3	20.9 ±1.3	20.8 ±1.3	21.0 ±1.3	21.0 ±1.3
after 64 days	32.0 ±2.8	31.0 ±3.1	30.3 ±3.7	31.5 ±3.0	31.4 ±2.9	30.8 ±2.8	48.1 ±4.9
"t"-test (5%)	NS*1	—	NS	NS	NS	NS	S*2
Gain (g)	11.0	10.0	9.3	10.8	10.6	9.8	27.1
Rate of feed intake (g fed/g B.W./day)	1.47	1.18	1.27	1.17	1.22	1.25	1.86
Feed efficiency (%)	45.7	52.0	45.0	54.8	52.3	47.8	74.8

*1 Non-significant at a 5% level, *2 Signficant.

Table 6. Effect of linolenic acid supplement in laurate diet on growth and feed efficiency of red sea bream.

Dietary lipid (%)	0*1	9	8.5	8	7	6	Pollock
Laurate 18:3 ω3	0	0	0.5	1	2	3	liver oil*2
No. of fish							
at start	20	20	20	20	20	20	20
dead	0	1	1	3	5	0	0
Ave. body wt. (g)							
at start	7.5 1.3	7.4 1.2	7.4 1.2	7.4 1.2	7.5 1.2	7.4 1.2	7.4 1.2
after 45 days	12.6 2.3	8.1 1.7	10.8 2.3	11.5 2.5	14.1 2.6	14.2 2.9	23.0 4.8
"t"-test (5%)	S	S	S	S	S	S	—
Gain (g)	5.1	0.7	3.4	4.1	6.6	6.8	15.6
Rate of feed intake	1.97	1.87	1.95	2.40	2.60	2.24	2.52
Feed efficiency (%)	55.7	11.8	40.9	45.2	57.8	61.6	88.5

*1 Fat-free diet, *2 Diet YR-1.

and feed efficiency of fish fed linolenic acid at a level up to 1 percent were greatly inferior to those of the fat-free diet group. Moreover, even the growth rate and feed efficiency of 2 percent and 3 percent linolenic acid groups were as poor as those of the fat-free diet group, in comparison with the fish fed pollock liver oil. Takeuchi and Watanabe (1977) demonstrated that elevated dietary laurate levels increased the requirements of rainbow trout for linolenate and that the maximum growth and feed efficiency were obtained at a 2 percent level of linolenic acid when the dietary laurate level was 8 percent. From these results, it can be concluded that linoleic and linolenic acid are not very important *per se* for the nutrition of red sea bream in contrast to terrestrial animals and freshwater fishes.

In an earlier study, (Yone, et al., 1971), pollock liver oil showed a high nutritive value for red sea bream, which is poor in the ω6 series fatty acids and linolenic acid, but is rich in ω3 highly unsaturated fatty acids (HUFA) with more than 20 carbon atoms, as shown in Tables 3 and 4. Hence, Yone and Fujii (1975) supposed that the essential fatty acids of red sea bream may be HUFA, and examined the effect of HUFA on the growth rate and feed efficiency of red sea bream. HUFA was prepared from cuttle-fish liver by saponification, methylesterification, and concentration by urea fraction technique, followed by distillation under vacuum. The HUFA preparation contained mainly eicosapentaenoic (20:5 ω3) and docosahexaenoic acid (22:6 ω3) and was free from linolenic acid and ω6 fatty acids. Consequently, the nutritive value of corn oil was remarkably improved by the supplementation of HUFA, and the fish fed HUFA diets exhibited high growth and feed efficiency comparable to the pollock liver oil diet group (Yone and Fujii, 1975). Furthermore, in the experiment using the laurate diet (Fujii and Yone, 1976), the growth rate and feed efficiency of fish fed the HUFA supplemented diet were extremely superior to those of the linolenic acid supplemented diet group, and were comparable to those of the groups fed the diets containing pollock liver oil and a combination of corn oil and HUFA. Red sea bream also showed markedly low growth and feed efficiency by the feeding of the diet containing tallow with a comparatively high melting point and low levels of ω6 and ω3 fatty acids, as shown in Table 7. However, the HUFA supplementation extremely improved the nutritive value of tallow (Yone and Takahashi, 1977).

From the latter three experiments, it was verified that HUFA have an essential role in the nutrition of red sea bream. Therefore, the amount of HUFA and 20:5 ω3 required by red sea bream was subsequently determined by a 50 and 60 day feeding trial (Fujii, et al., 1976; Nakayama and Yone, 1977). These fatty acids were supplemented to the corn oil diet at five levels. As shown in Figure 1, the growth rate and feed efficiency were improved with the increase in dietary HUFA and 20:5 ω3 up to 0.5 percent level. From the relationship between dietary fatty acid levels and growth of fish or feed efficiency, the requirement of red sea bream for HUFA and 20:5 ω3 is estimated at over 0.5g per 100g diet. It can be considered that 20:5 ω3 is also one of the essential fatty acids of red sea bream.

Table 7. Effect of ω3 highly unsaturated fatty acids (HUFA) supplemented at a 0.5% level into tallow and corn oil diet on the growth and feed efficiency of red sea bream.

Dietary lipid	Tallow (9%)	Tallow (8.5%) HUFA (0.5%)	Corn (8.5%) HUFA (0.5%)	Pollock 9%, YR-6)
No. of fish				
at start	22	21	21	21
dead	9	6	7	2
Ave. body wt. (g)				
at start	19.5±2.5	19.4±2.6	19.5±2.4	19.5±2.5
after 64 days	21.9±2.6	32.7±7.2	33.6±7.2	32.1±6.7
"t"-test (5%)	S	NS	NS	—
Gain (g)	2.4	13.3	14.1	12.6
Feed efficiency (%)	66.8	80.2	95.3	96.5

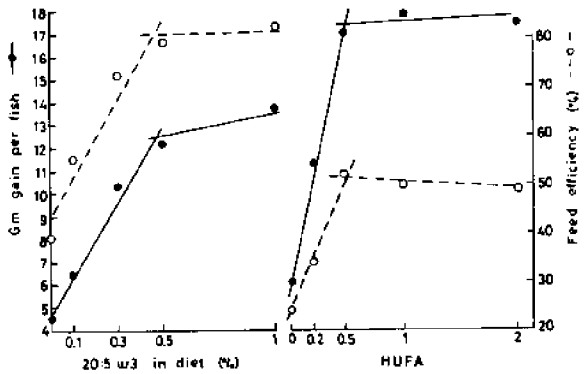

Figure 1. Relationships between growth of fish or feed efficiency and dietary 20:5 ω3 or HUFA levels.

If the different essential fatty acid requirement of red sea bream from freshwater fish and terrestrial animals was due to continual feeding on fodder with a different fatty acid composition, it can be presumed that fish with feeding habits similar to that of red sea bream would have a similar requirement for essential fatty acids, as well as the converse. So, Yamada and Yone (1977) examined the essential fatty acids of opaleye (*Girella nigricans*), a semi-herbivorous fish, black sea bream (*Mylio macrocephalus*), an omnivorous fish, and yellowtail, an extremely carnivorous fish.

In the growth and feed efficiency of opaleye, which often feed on seaweed, there was no effect of linolenic acid supplementation to a tallow diet, but the supplementation of 20:5 ω3 and HUFA improved markedly the nutritive value of tallow comparable to that of pollock liver oil (Table 8). On the other hand, linoleic acid supplementation did not elevate the growth rate, but elevated considerably the feed efficiency, differing from red sea bream (Yamada and Yone, 1977; Yone, 1978).

Black sea bream also exhibited a similar requirement for essential fatty acids as that of opaleye (Table 9). Namely, the supplementing of 20:5 ω3 and HUFA with a tallow diet at a 1 percent level greatly elevated the growth and feed efficiency. These parameters were appreciably elevated with linoleic acid but not with linolenic acid (Yamada and Yone, 1977; Yone, 1978). Accordingly, the essential role of ω6 series fatty acid, especially 20:4 ω6, in the nutrition of opaleye and black sea bream should be studied in the near future.

In the yellowtail which is an extremely carnivorous fish and is the most important culture fish in Japan, the supplementation of 20:5 ω3 and HUFA at a 1 percent level improved considerably the nutritive value of tallow and reduced the mortality. On the other hand, the

growth rate of the groups fed linoleic and linolenic acid was similarly low in the tallow diet group, with a higher mortality after 20 days of feeding (Table 10).

Table 8. Effects of linolenic acid (18:2), linolenic acid (18:3), eicosapentaenoic acid (20.5%), and HUFA supplemented at a 1% level into tallow diet on growth and feed efficiency of opaleye.

Dietary lipid	Tallow	18:2	18:3	20:5	HUFA	Pollock*[1]
No. of fish						
at start	30	30	30	30	30	30
dead	6	2	2	2	7	8
Ave. body wt. (g)						
at start	5.4	5.5	5.5	5.5	5.5	5.5
	±0.8	±0.9	±0.8	±0.9	±1.0	±0.8
after 62 days	9.4	11.0	9.9	12.9	13.0	16.5
	±2.3	±3.0	±2.1	±3.7	±3.8	±4.5
"t"-test (5%)	—	S	NS	S	S	S
Gain (g)	4.0	5.5	4.4	7.4	7.5	11.0
Rate of feed intake*[2]	2.80	2.30	2.48	2.32	2.63	2.26
Feed efficiency (%)	33.4	50.5	39.9	60.4	54.1	77.1

*[1]YY-2.

*[2]FX100/ $\frac{Wo+Wt}{2}$ or $\frac{No+Nt}{2}$ • t

Table 9. Effects of 18:2, 18:3, 20:5 and HUFA supplemented at a 1% level into tallow diet on the growth and feed efficiency of black sea bream.

Dietary lipid	Tallow	18:2	18:3	20:5	HUFA	Pollock*
No. of fish						
at start	20	20	20	20	20	20
dead	0	3	8	0	0	0
Ave. body wt. (g)						
at start	10.8	10.9	11.1	11.0	10.8	10.9
	±2.1	±2.4	±2.6	±2.4	±2.3	±2.4
after 72 days	20.6	24.4	22.8	31.0	27.2	31.2
	±5.4	±6.8	±9.2	±8.4	±7.2	±6.9
"t"-test (5%)	S	S	S	NS	NS	—
Gain (g)	9.8	13.5	11.7	20.0	16.4	20.3
Feed efficiency (%)	39.2	45.9	37.6	67.7	58.2	69.6

*YR-6.

Table 10. Effects of 20:5 and HUFA supplemented at a 1% level to tallow diet on the growth and feed efficiency of yellowtail.

Dietary lipid	Tallow	20:5	HUFA	Pollock*
No. of fish				
at start	30	30	30	30
dead	20	12	13	4
Ave. body wt. (g)				
at start	25.3±5.0	24.8± 5.0	24.9±5.2	25.0± 5.3
after 31 days	37.2±9.5	57.5±15.5	66.0±9.8	83.5±23.5
"t" test (5%)	—	S	S	S
Gain (g)	11.9	32.7	41.1	58.5
Feed efficiency (%)	24.0	56.8	71.8	83.9

* YY-2.

Recently, with turbot, Cowey, et al. (1976b), demonstrated that the substitution of a part of oleic acid with 1 percent cod liver oil resulted in the best growth after 16 weeks feeding, followed by that with linolenic acid, 20:4 ω6, and linoleic acid in decreasing order.

On the basis of these findings, it can be asserted that a species specificity also exists in the essential fatty acid requirement of marine fish, but that linolenic acid is not so important for the nutrition of marine fishes as for freshwater fishes and HUFA are essential for fatty acid metabolism of marine fishes.

Conversion of Linolenic Acid to ω3-highly Unsaturated Fatty Acids in Marine Fishes

A reason for the difference observed in the essential role of linolenic acid between marine and freshwater fishes can be presumed from changes in fatty acid composition noted during a long term feeding with a linolenic acid supplemented diet. Namely, in rainbow trout (Castell, et al., 1972b; Yu and Sinnhuber, 1972; Watanabe, et al., 1974) and carp (Watanabe, et al., 1975), the percentage of HUFA in body lipid increased by the feeding of linolenic acid. However, it was reported that linoleic and linolenic acid in the diet do not undergo chain elongation and further desaturation in plaice (*Pleuronectes platessa*) (Owen, et al., 1972) and turbot (Cowey, et al., 1976b). In red sea bream (Fujii, et al., 1976), also, the level of HUFA in liver phospholipid did not increase even if linolenic acid was supplemented to the corn oil diet at a 4 percent level (Table 11). Accordingly, it is supposed that marine fishes possess lower abilities to convert linolenic acid to HUFA when compared with freshwater fishes.

Table 11. Percentage fatty acid composition of phospholipid from liver of red sea bream fed corn oil diet with linolenic acid at several levels.

	Corn oil	0	9	8.5	8	7	5
Dietary lipid (%)	18:3 ω3	0	0	0.5	1	2	4
ω3 series fatty acids	18:3	tr	0.5	1.1	1.7	3.1	6.1
	20:5	3.4	2.1	2.0	1.7	2.3	2.7
	22:5	1.3	1.1	1.3	1.2	1.3	1.3
	22:6	7.2	6.3	7.0	5.5	8.6	8.2
	20<	11.9	9.5	10.3	8.4	12.2	12.2
ω6 series fatty acids	18:2	6.1	21.8	24.1	21.6	22.1	20.1
	18:3	tr	1.3	1.3	1.2	0.9	0.8
	20:2	3.6	1.3	0.8	1.2	0.7	0.8
	20:3	0.3	3.3	3.2	2.8	2.6	1.9
	20:4	0.6	0.6	0.6	0.7	1.0	1.5
	20<	4.5	5.2	4.6	4.7	4.3	4.2

Recently, the study by Owen, et al. (1975), confirmed this supposition. ^{14}C-labelled linolenic acid was orally administered to turbot and rainbow trout, after pre-feeding with a fat-free diet. On the basis of the radioactivity of each fatty acid at the sixth day after the administration, it was found that about 83 percent of linolenic acid was converted in rainbow trout to HUFA, mostly to 22:6 ω3, as compared to only 15 percent in turbot. Yamada, et al. (1980), also compared the conversion of linolenic acid to HUFA in red sea bream, black sea bream, and opaleye with that in rainbow trout, using the same method employed by Owen, et al. (1975). However, in the pre-feeding, a diet containing pollock liver oil was used.

As shown in Table 12, the radioactivity of 22:6 ω3 fraction exhibited 14.5 percent of total activity in rainbow trout, but 1.1 to 4.9 percent in marine fishes.

From these findings, it is postulated that the slow conversion of linolenic acid to HUFA in marine fishes is a cause of the difference observed in the essential role of linolenic acid between marine fishes and freshwater fishes.

Table 12. Distribution of ^{14}C into fatty acids in total lipid of marine fishes and rainbow trout fed [1-14C] linolenic acid. Values are expressed as percent of the total radioactivity of tissue lipid.

Fatty acid	Black sea bream	Opaleye	Striped mullet	Rainbow trout
14:0	0.2	0.1	0.5	3.0
16:0 16:1	0.5	0.4	0.5	1.4
18:0 18:1	0.2	0.4	0.2	3.4
18:3 ω3	82.2	79.9	83.4	38.8
18:4 ω3	8.3	7.7	6.9	3.1
20:3 ω3	3.0	0.9	2.4	10.6
20:4 ω3	0.4	tr	0.3	4.3
20:5 ω3	0.1	0.7	1.1	2.6
22:5 ω3	tr	0.7	0.2	7.9
22:6 ω3	1.1	4.9	1.6	14.5

Water temperature: Seawater 23.3—27.0°C; Freshwater 24.4—26.1°C.

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Bristol Bay Sockeye Salmon (*Oncorhynchus nerka*) — An Exploration in Factors Influencing Saltwater Growth

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Abstract

A preliminary investigation was undertaken to determine the effects of sea surface temperature, population size and weather factors on saltwater growth of Bristol Bay sockeye salmon. From the files of scales collected in the past, 815 scales taken from female fish were sampled and analyzed. Scales were from those fish that migrated to sea in 1965, 1966, and 1967 (considered "warm years"), and 1970, 1971, and 1972 (considered "cold years"). Numbers of scales used were 300 age 5_3 (2.2) and 133 age 6_3 (2.3) fish for the Kvichak River, and 293 age 5_3 (2.2) and 89 age 6_3 (2.3) fish from the Ugashik River. For each saltwater growth zone, the width was measured and the number of circuli within each zone was counted. Total saltwater growth and total number of circuli were significantly related to fish length and significantly related to one another. Width measurements were regressed on mean annual sea surface temperature, population size (an index based on numbers of returning adults) and, for first year's saltwater growth, weather data from the Pribilof Islands. First year's growth was significantly related to temperature for the two age groups combined and the two stocks significantly differed from one another.

Examining first year's growth in 5_3 fish separate from 6_3 fish showed that fish from the Ugashik River grew more than Kvichak River fish and the regression coefficients were 0.55 for Ugashik 5_3 fish and 0.41 for Kvichak 5_3 fish. Second year's growth showed no consistent patterns of temperature-growth relationships. There was a significantly larger growth zone for 5_3 than for 6_3 age fish within rivers but this difference was not significant in all years. Second year's growth within age groups between rivers was not significantly different. Third year's growth and plus growth showed no significant relation to temperature. There was no significant difference in third year's growth between the two stocks. Plus growth was significantly larger in 5_3 than in 6_3 age fish. A multiple regression analysis, correlating SST and population size with the various years of ocean growth of 5_3 aged fish showed that a significant amount of variation in growth was accounted for by the addition of population size. The R values were: 0.568 for Ugashik fish and 0.413 for Kvichak fish in the first year; 0.434 for Ugashik fish and 0.305 for Kvichak fish in the second year. Results for third year and plus growth were inconclusive. Including weather variables in the multiple correlation equation for first year's growth of age 5_3 fish, precipitation was the only variable that accounted for a significant amount of variation in growth. The results are discussed in terms of possible mechanism and significance to salmon enhancement programs.

Introduction

Environmental changes play a significant role in fluctuations of fish abundance in the oceans, but the causal mechanisms are imperfectly known. For many years it had been hypothesized that differential mortality during the "critical period" of first feeding determines variations in year class strength. This "critical period" is determined by the relation between the timing of larval fish hatching and food of the correct species, size, concentration, and availability. Research indicates that this hypothesis is correct (May, 1974). As an example, Lasker (1978) demonstrated that short term changes in oceanographic stability, and in turn the meteorologic conditions, influenced the survival of anchovy larvae off the coast

of California. The mechanism by which this occurred was the water's turbulent conditions which broke up food concentrations when fish first began feeding, making it difficult for the larval fish to obtain enough food to survive. Emphasis is being placed on research similar to Lasker's (1978) which will elucidate how short term changes of air-sea interactions influence the early life stage of pelagic fish. However, because salmon do not exist in a pelagic larval stage, the more long term environmental changes occurring between years and the biological consequences would seem to be more important.

One factor which influences many aspects of fish ecology is water temperature, influencing production of fish food organisms (Cushing, 1975; Cushing and Dickson, 1976), fish distribution (Hubbs, 1948; Radovich, 1961) and migration (Tully, et al., 1960; Jakobsson, 1969). Temperature has also been shown repeatedly to be one of the most influential factors in fish growth (Brett, et al., 1969). In the north Pacific Ocean there has been a long term decline in sea surface temperature, although very slight (Ricker, et al., 1978). However, of more significance and possibly having more impact are the relatively short term changes which have occurred within a several year period. For example, in both the Gulf of Alaska and Bering Sea, 1967 was one of the warmest years on record whereas 1971 was one of the coldest (Straty and Jaenicke, in press; H. J. Niebauer, personal communication). Mean annual sea surface temperature in 1967 for the Gulf was 7.5°C and for the Bering Sea 5.4°C, whereas in 1971 the temperature was 6.3°C for the Gulf and 3.2°C for the Bering Sea. Straty and Jaenicke compared the first year's growth of Bristol Bay sockeye salmon (*Oncorhynchus nerka*) for these two different periods. Using number of circuli, the width of the first year's saltwater growth, and three other features of the scale, they found significant differences for all five features between the two years. They concluded more growth occurred in the warm year of 1967.

Although not well understood, density-dependent growth appears to be quite common in fishes (Hepher, 1967; Cushing, 1975; Ware, 1980), particularly in the immature phase of life history. Density-dependent growth has been shown to occur in the freshwater stage of sockeye salmon (Hartman and Burgner, 1972) and the size of returning adults also appears to be density dependent; mature fish during years of peak numbers (every five years with Bristol Bay stocks) are smaller than those during non-peak years (C. P. Meacham, personal communication). Growth in fish is a sensitive index to resource availability (Werner and Hall, 1977), i.e., since growth is plastic it will increase with a relative increase in food availability and decrease when food is scarce; and it has been assumed that density-dependent growth in Bristol Bay sockeye salmon is a reflection of resource availability due to intraspecific competition for food (Tsuneo Nishiyama, personal communication). Food and temperature also interact in their influence on growth. Brett, et al. (1969) have shown this for young sockeye salmon, growth efficiency being different for different food levels and temperature combinations.

Before an extensive study is begun into the effects of oceanic conditions on ocean growth of Bristol Bay sockeye salmon, a preliminary study was initiated under the hypothesis that temperature of a relatively warm period (1965 to 1967) and a relatively cold period (1970 to 1972), and population size will influence oceanic growth. An initial examination was also made of the possible influence of weather conditions on first year's growth. This paper presents the results of this preliminary study.

Methods and Materials

The Alaska Department of Fish and Game (ADF&G) has been sampling and storing Bristol Bay sockeye salmon scales since the 1950s. They were obtained by the "key scale" method (Tesch, 1968), in this case from the left side of the fish, two scales above the lateral line and

below the posterior insertion of the dorsal fin. For this study, because there are differences between stocks and sexes in Pacific salmon due to genetic characteristics (Ricker, 1972), scales taken off of escapement females from two river systems were sampled from the scale files of ADF&G. A total of 815 scales were sampled, 300 age 5_3 fish[1] and 133 age 6_3 fish from the Kvichak River, and 293 age 5_3 fish and 39 age 6_3 from the Ugashik River. Scales sampled were those that did not show appreciable resorbtion and were taken from fish which migrated to sea as smolts in 1965, 1966, 1967, 1970, 1971 and 1972. Numbers of scales used for each age group, for each river and year are given in Table 1. The first three years were considered "warm years" and the latter three "cold years" as determined from mean annual sea surface temperatures (1967 was the warmest year on record and 1971, one of the coldest). Impressions of scales were made on acetate plastic (Arnold, 1951) and projected at a magnification of 100X on a projector which is described by Ryan and Christie (1975). For each scale the following measurements were obtained as measured from 20° ventral to the longest scale axis:

1) The width of the freshwater growth zone from the focus to the last circulus which was laid down during freshwater growth.
2) The number of circuli in and width of each year's saltwater growth zone. These were measured from the last circulus in the previous growth zone to the last circulus formed in the first, second, or third (for 6_3 fish) saltwater growth zones.
3) The number of circuli in and width of the plus growth zone as measured from the last circulus in the second (for 5_3 age fish) or third (for age 6_3 fish) year's growth to the scale edge.

Table 1. Sample size for each age group from the Ugashik and Kvichak Rivers which migrated to sea during the years used in this study. Note that a 5_3 fish which migrated to sea in 1965 would return to spawn in 1967, whereas a 6_3 fish which migrated at the same time would have returned in 1968.

		Year of Migration						
River	Age[1]	1965	1966	1967	1970	1971	1972	N
Ugashik	5_3	50	50	50	50	42	51	293
	6_3	8	3	3	50	5	20	89
Kvichak	5_3	50	50	50	50	50	50	300
	6_3	50	3	0	50	4	26	133

[1]The Gilbert-Rich system of aging is used in this study. Age 5_3 fish is equivalent to a 2.2 age fish in the European system. Similarly 6_3 fish is equivalent to a 2.3 age fish.

Total distance of the saltwater growth zones was then correlated with the total number of circuli. For Ugashik fish $r = 0.789$ (d.f., = 1,380; $p << 0.001$) and for Kvichak fish $r = 0.734$ (d.f., = 1,431; $p << 0.001$). Therefore, distance of the various saltwater growth zones were used in the analysis of the influence of various factors on growth.

Environmental data were obtained from two sources. Monthly mean sea surface temperatures (SST) were provided by Dr. D. R. McLain (NMFS, Fleet Numerical Oceanography Center, Monterey, California) for one point in the Bering Sea (57°N, 170°W) near the Pribilof Islands and one just northeast of Kodiak Island (58°N, 150°W). Following Straty and Jaenicke (in press), monthly mean sea surface temperatures were plotted and the area under the resultant curve determined, herein called "temperature." Temperatures calculated for the Bering Sea were correlated with first year's growth, whereas the temperatures calculated for the Gulf of Alaska were correlated with second and third (for 6_3

[1]The Gilbert-Rich system of aging is used in this study. An age 5_3 fish is equivalent to a 2.2 age fish in the European system. Similarly, 6_3 fish are equivalent to age 2.3 fish.

fish) year's growth. Temperatures correlated with plus growth, since maturing fish spend time both in the Gulf and Bering Sea during their spawning migration, were determined by plotting monthly mean SST from the Gulf for January through April, and monthly mean from the Bering Sea for May and June. Weather data, monthly means of wind cubed (an index of water mixing, Forest Miller, personal communication), precipitation, degree days and cloud cover, were obtained from the National Weather Service for St. Paul Island (Pribilof Islands). For each weather parameter an overall mean for the months of June through December was calculated and correlated with first year's growth.

To determine the effect of population size on growth, it is necessary to have numbers of migrating smolts. However, adequate information does not exist because of inaccuracies in smolt estimation (C. P. Meacham, personal communication). Therefore, an index of population size was developed using numbers of returning adults from all major river systems of Bristol Bay. For correlation with first year's growth, the index was developed by summing the numbers of adults which went to sea at the same time irrespective of when they returned to spawn. For second and third year's growth, the index was developed by summing the number of adults which would have been in the Gulf of Alaska during the years of interest irrespective of when they returned to spawn. The index correlated with plus growth was the number of adults which returned in the year of interest irrespective of age.

The relationship between growth measurements, population index, and physical parameters was determined by univariate regression analysis, analysis of covariance, with temperature as the covariate, and stepwise regression analysis (Steele and Torrie, 1960; Draper and Smith, 1966). Computations were performed using BMDP programs (Dixson and Brown, 1979). When mean growth is presented in the text, plus or minus one standard error is also given.

Results

Temperature

Table 2 summarizes the results of a covariance analysis of first year's growth for age 5_3 and 6_3 fish from two rivers. Temperature was the covariance. There was a significant response to temperature for both Kvichak River and Ugashik River stocks. There were no differences in mean growth for the two age groups. The regression coefficients for the age groups combined are not that strong, but they are significant. Considering the 5_3 age groups alone, the regression coefficients are much stronger than when 5_3 and 6_3 fish are considered as a group, 0.55 for Ugashik River fish and 0.41 for Kvichak River fish (Table 3). Comparing age 5_3 fish from the two river systems, covariance analysis showed that the two age groups responded differently to temperature (d.f. = 1,589, p = 0.035). Therefore, separate regression analyses were performed and the results are summarized in Table 3. Ugashik fish had a greater response and a stronger correlation than Kvichak fish. A t-test was used, because of the dif-

Table 2. Summarized results of comparing first year's growth of all fish from the Ugashik and Kvichak Rivers. Analysis of covariance was used in the comparisons with temperature as a covariate. P = probability values, d.f. = degrees of freedom and r = regression coefficient between temperature and growth for age 5_3 and 6_3 fish combined.

	Kvichak		Ugashik	
Test	d.f.	P	d.f.	P
Equal Slopes	1,429	0.348	1,378	0.305
Slope = 0	1,430	0.001	1,379	$<<0.001$
Equal Adjusted Means	1,430	0.946	1,379	0.471
r	0.36		0.44	

Table 3. Summarized results of regression analysis and t-test for first year's growth of aged 5_3 from the Ugashik and Kvichak Rivers. b = slope of regression line. See Table 2 and text for further explanation.

	Ugashik	Kvichak
	Regression Analysis	
r	0.55	0.41
b	307.99	223.32
d.f.	1,291	1,298
P	$<<0.001$	$<<0.001$
	Test of Means	
Mean Growth ± S.E.	370 ± 2.40	363 ± 2.36
P	0.028	

ferent response to temperature, to determine if there was a difference in mean growth. Table 3 shows that Ugashik fish grew more than Kvichak River fish. Even though sample sizes for 6_3 fish are small for both rivers or nonexistent in one year, covariance analysis was performed and showed there was a significant response to temperature (d.f. = 1,219; p = 0.015), no difference in the growth-temperature relationship between the two stocks (d.f. = 1,218; p = 0.646) and no difference in mean growth (d.f. = 1,219; p = 0.348; Ugashik $\bar{x}$ = 365 ± 3.89, Kvichak $\bar{x}$ = 361 ± 3.18).

Second year's growth for the four groups, 5_3 and 6_3 aged fish from both river systems, were compared by analysis of covariance and there was a significant difference between slopes (d.f. = 3,807; p = 0.031). Various combinations of age groups from the two river systems were compared by covariance analysis and only one comparison, 6_3 fish from Ugashik and Kvichak Rivers, showed that they had similar slopes (d.f. = 1,218; p = 0.073). The analysis also showed that there was no relationship between growth and temperature (d.f. = 1,219; p = 0.197), with mean growth the same for the two groups (Ugashik $\bar{x}$ = 296 ± 5.0 and Kvichak $\bar{x}$ = 294 ± 4.08; d.f. = 1,219; p = 0.646). Thus, age 6_3 fish from the two river systems grow similarly in the second year and temperature did not significantly affect growth.

Table 4 summarizes the results of regression analysis between growth and temperature for the groups separately. In contrast to first year's growth, there was only one significant relationship to temperature. However, this was negative and is undoubtedly due to a small sample size. To determine whether there was a significant difference in growth between 5_3 and 6_3 fish within river systems, t-tests were performed for those years in which there were adequate samples (see Table 1), and the results are shown in Table 5. For both river systems, 5_3 fish grew significantly more than 6_3 fish for the years pooled. However, this significant difference is not consistent over years and for these Ugashik fish which migrated to sea in 1970, growth was essentially identical (Table 5). Comparing 5_3 age fish from the two rivers by t-test showed that there was no significant difference in growth (d.f. = 592; p = 0.146; Ugashik $\bar{x}$ = 320 ± 2.8; Kvichak $\bar{x}$ = 314 ± 2.8). However, in 1971 there was a significant difference (d.f. = 90; p = 0.010) between the Ugashik 5_3 fish ($\bar{x}$ = 334 ± 5.9) and Kvichak 5_3 fish ($\bar{x}$ = 309 ± 6.8).

Table 4. Summary of regression analysis between second year's growth and temperature for the four groups of fish. See Tables 2 and 3 for further explanation.

	Kvichak		Ugashik	
	5_3	6_3	5_3	6_3
r	0.081	-0.186	-0.094	0.037
b	43.4	-188.9	-50.69	28.94
d.f.	1,298	1,131	1,291	1,87
P	$0.25>P>0.10$	$0.05>P>0.01$	$0.10>P>0.05$	$0.50>P>0.25$

Table 5. Second year's growth (means ± one standard error) for each age group within rivers and probability of means being different. Only those years in which an adequate sample size for 6_3 aged fish were used. Sample size in parentheses. See text for further explanation.

	Ugashik			Kvichak		
	Age			Age		
Year	5_3	6_3	P	5_3	6_3	P
1965	—	—		315 ± 6.1 (50)	309 ± 5.7 (50)	0.440
1970	299 ± 5.3 (50)	298 ± 5.7 (50)	0.922	302 ± 5.7 (50)	292 ± 6.1 (50)	0.217
1972	336 ± 5.5 (51)	300 ± 9.5 (20)	0.001	329 ± 6.4 (50)	273 ± 2.1 (26)	< < 0.001
Years Pooled	318 ± 4.2 (101)	299 ± 4.8 (70)	< < 0.001	315 ± 3.6 (150)	294 ± 4.3 (126)	< < 0.001

In comparing the growth over all years of 6_3 fish, a t-test showed there was no significant difference (d.f. = 220; p = 0.813; Ugashik $\bar{x}$ = 296 ± 4.8; Kvichak $\bar{x}$ = 295 ± 4.2).

For the third year's growth, even though sample size for some years is small (Table 1), an analysis of covariance was performed to determine if a temperature effect could be detected and whether there was a difference in growth between the two stocks. The analysis showed no differential temperature response (d.f. = 1,218; p = 0.714), with the slope of the relationship not significantly different from zero (d.f. = 1,219; p = 0.102). Also the two stocks grew similarly (d.f. = 1,219; p = 0.642), mean growth for Ugashik fish being 239 ± 4.19 and that for Kvichak fish 237 ± 3.44.

Covariance analysis of plus growth showed a significant difference between slopes for the four groups of fish, i.e., the two age groups within the two river systems (d.f. = 3,807; p = 0.007). Table 6 summarizes the results of the regression analysis between plus growth and temperature for the four groups. Two of the groups, Kvichak 5_3 and Ugashik 6_3, are marginally significant at the five percent level. However, their slopes are of opposite direction. The other two groups also have slopes of opposite sign and are not significant. It would seem then, since the results are inconsistent, that there is not a significant effect of temperature on growth during the last few months prior to spawning.

Table 7 summarizes results of several t-tests performed to determine whether there were differences in plus growth for the two age groups within and between the two river systems. For the years taken as a whole, there were no significant differences between 5_3 age fish from the two river systems nor between 6_3 age fish (Table 7). However, looking within years, differences did occur. For example, age 5_3 fish from the 1967 migrants, Ugashik fish had more plus growth than did Kvichak fish (113 ± 4.31 vs. 101 ± 3.91, respectively; d.f. = 98, p = 0.050), but the 1970 Kvichak migrants grew more in plus growth than those from 1970 Ugashik migrants (108 ± 3.81 vs. 92 ± 5.52, respectively; d.f. = 98, p = 0.017). For 6_3 age fish, 1970 migrants from Ugashik grew more than those from Kvichak (65 ± 3.68 vs. 53 ± 3.74,

Table 6. Summary of regression analysis between plus growth and temperature for the four groups of fish. See Table 4 for explanation.

	Kvichak		Ugashik	
	5_3	6_3	5_3	6_3
r	-0.114	-0.121	0.114	0.210
b	-115.22	-94.01	108.00	174.39
d.f.	1,298	1,131	1,291	1,87
P	0.049	0.19	0.053	0.049

Table 7. Plus growth (means ± on S.E.) for Ugashik and Kvichak 5_3 and 6_3 age fish and probability of means being different. See Table 3 for explanation.

	Mean (± S.E.)	d.f.	P
Ugashik 5_3	123 ± 2.39	591	0.363
Kvichak 5_3	126 ± 2.50		
Ugashik 6_3	58 ± 2.38	220	0.588
Kvichak 6_3	60 ± 3.05		
Kvichak 5_3	116 ± 2.60	274	$<<0.001$
Kvichak 6_3	58 ± 2.46		
Ugashik 5_3	114 ± 4.37	169	$<<0.001$
Ugashik 6_3	58 ± 3.26		

respectively; d.f. 98, p = 0.029) whereas the 1972 migrant Kvichak fish grew more than did Ugashik fish (67 ± 5.09 vs. 42 ± 5.24, respectively; d.f. 44, p = 0.001). Comparing 5_3 age fish with 6_3, 5_3 fish grew twice as much as 6_3 fish in their plus growth.

Temperature and Population Size

Table 8 summarizes the results of a stepwise regression analysis with temperature and population size as independent variables. For first year's growth, temperature entered the equation first in all cases and, as previously pointed out, R was significant at the p = 0.05 level or higher in three of the four groups. From the partial F-test the amount of additional information accounted for by adding population size was significant only for Ugashik 5_3 fish (F = 7.71; d.f. = 1,290; $p<0.01$). For Kvichak 6_3 fish the R value was not significant at the p = 0.05 level with both variables included.

In second year's growth, population was the first variable to enter for both 5_3 age groups and Ugashik 6_3. However, the R value was significant at p = 0.05 level or better for only the 5_3 age groups and there was a negative slope for the Ugashik 6_3 fish. When temperature entered into the equation for the three groups, the regression coefficient was raised substantially, but partial F-tests showed that a significant amount of information was accounted for by adding temperature only in the 5_3 age groups (Kvichak: F = 12.42, d.f. = 1,297, $p<0.001$; Ugashik: F = 57.19, d.f. = 1,290, $p<0.001$). For Kvichak 6_3 fish, temperature entered first and, as previously noted, the R value was significant at the p = 0.05 level. However, a significant amount of information was not accounted for when population was added to the equation.

In the third year, temperature entered the equation first for Kvichak 6_3 fish and, as previously noted, the R value was not significant at the p = 0.05 level. Including population in the equation raised the R value but it did not add a significant amount of information, nor was the R value significant. Population entered the equation first for Ugashik 6_3 fish, with a negative slope and was significant at p = 0.01 level. When temperature entered, it did not account for a significant amount of information.

For plus growth, temperature entered the equation first for three of the four groups, but the R value was significant p = 0.05 only for 5_3 and 6_3 Ugashik fish. For Kvichak 6_3 fish the slope was negative, but not significant at the p = 0.05 level. For Kvichak 5_3 fish, population entered the equation first and the R value was significant p = 0.01 and the slope was negative. When the second variables entered the equations for the four groups, partial F-tests showed that none account for a significant amount of variation.

Table 8. Summarized results of stepwise regression analysis for the various years of growth with temperature (T) and population (P) as independent variables. R = regression coefficient and n = sample size.

	First Year				Second Year			
	Kvichak		Ugashik		Kvichak		Ugashik	
	5_3	6_3	5_3	6_3	5_3	6_3	5_3	6_3
n	300	133	293	89	300	133	293	89
R	T:0.409**	T:0.184*	T:0.552*	T:0.139	P:0.235**	T:-0.186*	P:0.168*	P:-0.051
	T + P:0.413**	T + P:0.186	T + P:0.568**	T + P:0.143	P + T:0.305**	T + P:0.224*	P + T:0.434**	P + T:0.160
	Third Year				Plus Growth			
R	—	T:0.086	—	P:-0.276**	P:-0.165**	T:-0.121	T:0.114*	T:0.210*
	—	T + P:0.112	—	P + T:0.277*	P + T:0.177**	T + P:0.141	T + P:0.114	T + P:0.278*

*Significant at the $0.05 > P > 0.01$ level.
**Significant at the $P < 0.001$ level.

Other Factors

To examine the potential effect of other variables on growth, a stepwise regression analysis was done using, besides temperature and population, cube of the wind, degree days, air temperature, precipitation and cloud cover as predictor variables. Because of the small sample size for age 6_3 fish in various years, these variables were regressed on first year's growth of age 5_3 fish only. Table 9 summarizes these results. Precipitation entered the equation after temperature for Kvichak 5_3 fish and accounted for a significant amount of variation in first year's growth (d.f. = 9,297; p = 0.003). Degree days entered the equation next but did not account for a significant amount of variation (d.f. = 1,296; p = 0.25). For Ugashik 5_3 fish temperature entered the equation first followed by population, and as previously shown, both accounted for a significant amount of variation in growth. Next to enter the equation was precipitation and it accounted for a significant amount of variation (d.f. = 1,295; $p << 0.001$). Including other variables in the analysis did not account for any significant amount of variation in growth for either group of fishes.

Table 9. Summarized results of stepwise regression analysis of first year's growth for 5_3 aged fish. The independent variables shown are temperature (T), population (P), precipitation (PR), and degree days (DD). R is the multiple regression coefficient.

	Step 1	2	3
Variable	T	Kvichak PR	DD
R	0.405	0.435	0.439
Partial F Ratio	58.59**	T: 64.12** PR: 9.14**	T: 53.97** PR: 6.62** DD: 1.35
Variable	T	Ugashik P	PR
R	0.552	0.568	0.60
Partial F Ratio	127.59**	T: 113.39** P: 7.71*	T: 25.78** P: 23.69** PR: 16.40**

*Significant at the $0.05 > P > 0.01$ level.
**Significant at the $P < 0.01$ level.

Discussion

From the analysis presented in this preliminary study, there were significant relationships between several factors and growth as well as significant differences between age groups and stocks. However, any conclusions based on age, because of the small sample size and no data in one year for age 6_3 fish, may be looked on with circumspection. Even though there were these small sample sizes, the age comparisons were done primarily as a guide to further investigations. Analysis of covariance of growth for the fishes' first year at sea showed that 5_3 and 6_3 fish responded similarly to temperature and the relationship was significant. There was a differential response to temperature between the two stocks, analyzed with 5_3 and 6_3 fish combined and 5_3 fish separately. Growth by Ugashik fish was more strongly related to temperature than Kvichak fish and they grew significantly more. Regressions between temperature and second year's, third year's and plus growth resulted in three out of ten significant (barely at the p = 0.05 level) R values. However, two significant values and one nonsignificant value were negative in sign. Therefore, it can be concluded that there was a significant temperature affect only on first year's growth.

Lander and Tanonaka (1964) showed that age 5_3 Bristol Bay sockeye grew more in their

second year at sea than 6_3 age fish. Helle (1979), for chum salmon (*Oncorhynchus keta*), showed that there was a significant relationship between age at maturity and second year's growth, earlier maturity being related to more growth in the second year. This suggested that faster growing fish mature earlier. In the present study, comparing second year's growth, t-tests showed that for all years combined, age 5_3 fish grew significantly more than 6_3 fish in their second year at sea. However, comparing within years shows that the differences are not consistent from year to year, with there being no difference between age groups in some years. J. J. Pella and H. W. Jaenicke (personal communication) back calculated growth for two stocks of Bristol Bay sockeye salmon and found that 5_3 and 6_3 age fish may be the same size at the end of their second year at sea.

A number of studies have investigated the relationship of marine environment and survival (Cushing and Dickson, 1976; Sutcliffe, et al., 1977; Parrish and MacCall, 1978; Helle, 1979). But other than Straty and Jaenicke's study (in press), few studies have examined the relationship between marine environment and growth. Helle (1979) correlated the number of circuli, used as an indication of growth, in the first and second growth zones of chum salmon with a number of environmental factors. He found significant correlations between first year's growth and mean summer and fall water temperatures (positive), mean summer cloud cover (negative), mean summer and fall air temperatures (positive), and mean summer and fall dew point (positive). As found in our study, Helle found no significant relationship between second year's growth and ocean temperature. Ricker, et al. (1978) examined the relationship between the final size of various pink salmon stocks (*O. gorbuscha*) and the long-term decline in ocean temperature. They found positive relationships with temperature but only accounted for 9 percent to 25 percent of the change in the size of pink salmon. They also found some significant positive relationships with salinity but questioned their validity.

In contrast to the use of univariate correlation analyses in other studies to investigate the influence of environmental factors on growth, we have used a stepwise multiple regression approach with the thought that the factors under investigation would not act independently. Helle (1979), in his study of chum salmon, used the number of returning adults of a cohort as an index of population size and found no relationship between population size and growth in either the first or second year of ocean life. In the present study, using 5_3 fish in the analysis, population size accounted for a significant amount of additional variation in first year's growth for only Ugashik fish. However, even though it was significant, it raised the R value by only a small amount (0.016). This, in conjunction with Helle's results, may suggest that the number of migrating smolts does not influence first year's growth to any great extent and the temperature of the Bering Sea is more important. For second year's growth, population entered the equation first for both stocks and was significant at the $p = 0.05$ level for Ugashik fish and $p = 0.001$ level for Kvichak fish. Temperature entered the equation next for both stocks and accounts for a significant amount of variation in both cases (Table 8). The R value was raised from 0.235 to 0.305 for the Kvichak fish and 0.168 to 0.434 for the Ugashik fish. This indicates, recalling that univariate regression values between second year's growth and temperature were of opposite signs and not significant, there is an interaction between the two variables in influencing growth. It is interesting to note an unexpected result of the analysis. Regression values between temperature and index of population size used in the regression analysis of first and second year's growth had values varying from 0.626 to 0.819 (significant at the $p = 0.001$ level), implying that ocean survival is temperature dependent. Finally, environmental factors were included in the equation for first year's growth (Table 9). For Kvichak fish, precipitation accounted for a significant amount of variation in growth after temperature; whereas the third variable to enter (degree days) and all variables thereafter, including population, did not account for a significant amount of variation in growth. For Ugashik fish, besides temperature, population and precipitation accounted for significant amounts of variation in growth, whereas no other variables did, suggesting that

climatic factors may not influence first year's growth. This is in contrast to Helle (1979) who found significant relationships between first year's growth in chum salmon and mean summer and fall air temperature, mean summer and fall dew point and mean summer cloud cover, suggesting that climatic factors might influence growth.

Because of the implications to the enhancement of sockeye salmon and possibly other salmon, it will prove interesting to speculate on the reason for the results in this study. In this discussion it is assumed that the regression values between growth, temperatures and population size are reflecting valid biological relationships and not fortuitous ones, as the significant regression with precipitation may well be (the authors cannot attach any real biological meaning to a significant amount of variation in growth being accounted for by precipitation). Food organisms utilized by sockeye salmon have been studied and reviewed by Straty (1974), LeBrasseur (1972 and 1966), and French, et al. (1976). Generally, the tendency is for juveniles to feed on small organisms such as cladocerans, all stages of copepods and euphausiids, and larval fishes, whereas the maturing and immatures feed on larger organisms such as fish and squid. However, the maturing and immature fish do utilize euphausiids and amphipods, the proportions seeming to vary with season and geographic location. LeBrasseur (1972) classified various food organisms as herbivores, primary carnivores and secondary carnivores and concluded that maturing and immature sockeye (as well as pink and chum) salmon did not effectively utilize the spring bloom of primary consumers and that the energy represented by these organisms is stored by other elements higher in the food web, the larger carnivores. Thus, it is possible then, that the juveniles might more effectively utilize the large amount of biomass that exists during the summer and fall in the Bering Sea which is much greater than that occurring in the North Pacific (McAllister, 1961; LeBrasseur, 1965; Ikeda and Motoda, 1978; Iverson, et al., 1979). It is possible then that numbers of juveniles reaching the Bering Sea have not reached the point where competition comes into play and density-dependent growth effects are not exhibited. However, once the fish reach the North Pacific Ocean and begin utilizing organisms higher in the food web, density-dependent growth becomes important as the fish cannot effectively utilize the larger numbers of primary consumers. Several sources of information imply that this may be the case and that ocean limitation exists in other than the first year at sea. First are the results of the present study, population accounting for a significant amount of variation in growth for both stocks in the second year but not the first. Second is from two studies. Walters, et al. (1978), from a computer simulation model of growth and survival during the first six months of near shore inhabitation by major British Columbia salmon stocks, concluded that ocean limitation was unlikely unless only a small fraction of the total zooplankton production is available to salmon. Bailey, et al. (1975), investigating whether an estuary was near carrying capacity for pink and chum salmon, concluded that based on food organisms, the estuary could support ten times the number of young over that which they found. In contrast to these, Peterman (1978) presents evidence that in some stocks there is not an increase in adult returns with an increase in smolt abundance and that marine survival of some stocks is affected by other stocks or cohorts. Finally, the size of mature Bristol Bay sockeye (particularly 5_3 age fish) are known to be smaller during peak years (C. P. Meacham and Tsuneo Nishiyama, personal communication).

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Food of Lanternfishes in Suruga Bay, Central Japan

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Abstract

An examination was made of the stomach content of four species of lanternfishes, *Myctophum nitidulum*, *Myctophum orientale*, *Diaphus suborbitalis*, and *Benthosema pterota* collected from Suruga Bay on the Pacific coast of central Japan. The first two are epipelagic species migrating between the surface and 300 m, and the last two are mesopelagic species migrating between 20 m and 400 m.

Copepods were always predominant in the stomachs of these four species. There was a greater diversity of animals in the stomachs of the epipelagic species than in those of the mesopelagic species. Copepods, larvae of shrimps and crabs, euphausiids, amphipods, cirriped larvae, appendicularians, etc., were found in the stomachs of the epipelagic species, while copepods and euphausiids were the major components in the stomachs of the mesopelagic species. The difference in the diversity of zooplankton fauna between the shallower and deeper water layers would reflect in the stomach contents of the two groups.

The average stomach content index in weight was 1.13 in *M. nitidulum*, 0.89 in *M. orientale*, 0.86 in *D. suborbitalis*, and 0.74 in *B. pterota*. The stomach content index was higher in the epipelagic species than in the mesopelagic species.

It is suggested from stomach content analysis that the lanternfishes in Suruga Bay are positioned at a level between zooplankton and fishes in their trophic relationship, and thus play a significant role in the food web of the marine ecosystem in the bay.

Four species of lanternfishes, *Diaphus gigas*, *Diaphus sagamiensis*, *Diaphus watasei* and *Diaphus suborbitalis* are utilized as human food by local fishermen.

Introduction

Lanternfishes are known as deep-water micronekton widely and abundantly distributed in the oceans of the world. About 130 species of lanternfishes have been reported from the Pacific Ocean, and 37 of these have been found in Suruga Bay on the Pacific coast of central Japan. The author had opportunities to work on stomach analysis of four species of lanternfishes, *Myctophum nitidulum*, *Myctophum orientale*, *Diaphus suborbitalis*, and *Bentosema pterota* collected from Suruga Bay during the period 1969 to 1977. This paper deals with the stomach contents of these four species, describing the species of food animals and their abundance. It also includes a note on the utilization of several species of lanternfishes as human food.

Collection of Material

For collection of the epipelagic lanternfishes, a ring net, 130 cm in mouth diameter and 4.5 m in length, made of stramin, was towed through the surface layer of the sea at night. Seven tows were completed in November-December 1969 and two tows in November of 1970 by the R/V Tokaidaigaku Maru II, on a course from Miho Key to Osezaki in Suruga Bay. Mesopelagic lanternfishes were collected from the catch of sergestid seine boats operating

on the fishing grounds from Miho Key to the mouth of River Fuji, and also off Tagonoura and off Yaizu. Samples of lanternfishes were taken from the catch of sergestid seine nets six times in October-December 1975, five times in May-June 1976, three times in November-December 1976, and seven times in April-June 1977, for a total of 21 samplings (Figure 1). The sergestid fishing was always carried out at night, and the seine net was lowered to 150 m depth and then hauled. The following specimens obtained by the ring net and seine net were examined.

Epipelagic species:

Myctophum nitidulum Garman78 individuals

Myctophum orientale (Gilbert) 33 individuals

Mesopelagic species:

Diaphus suborbitalis Weber 571 individuals

Benthosema pterota (Alcock) 380 individuals

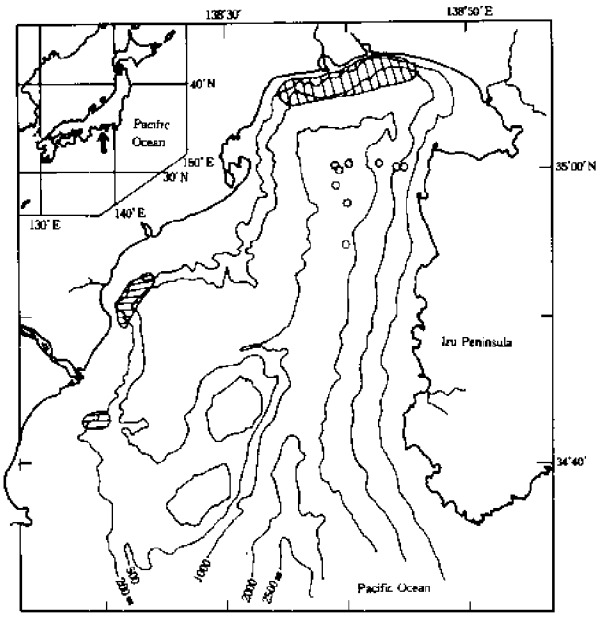

Figure 1. Index map for sampling in Suruga Bay.
: where two species of epipelagic lanternfishes were obtained in 1969-1970.
: where two species of mesopelagic lanternfishes were obtained in 1975-1977.
Inserted map shows Suruga Bay indicated by an arrow in large map.

Processing of Material

Specimens were immediately fixed with formalin, and taken back to the laboratory. Body length, body weight, and head length were measured. The number of soft rays of the dorsal fin and anal fin, number of anal luminous organs (AO) on both sides of the body, number of gill-rakers on the left first gill arch, vertebral number, and other meristic characters were recorded for species identification. The stomachs were dissected and the contents were weighed then preserved for later examination. Sex was determined by examining the gonads. Food animals contained in the stomachs were identified at the level of species, genus, or family, and the number of individuals was counted. From this, the stomach content index [(weight of animals taken/body weight of fish) x 100] was calculated.

Results

Food of *Myctophum nitidulum*

Copepods were found in 63 (80.8 percent in number of stomachs) of the 78 fish. Twenty-two genera of copepods in the stomachs of the fish were identified. Their abundance was in the descending order of *Eucalanus*, *Paracalanus*, *Oncaea*, *Euchaeta*, *Calanus*, and *Undinula*. Larvae of shrimps and crabs, in particular brachyuran zoea and megalopa, were also abundant. Euphausiids, amphipods, and cirriped larvae also occurred in the stomachs of the fish though in small numbers (Figure 2). The frequency distribution of the stomach content index showed that stomachs with stomach indices in the two classes: 0.001-0.200 and 0.201-0.400 occupied 30.8 percent of the total. The indices of the classes from 2.401 to 5.801 were 1.3 percent of the total. The average index was 1.13 (Figure 3).

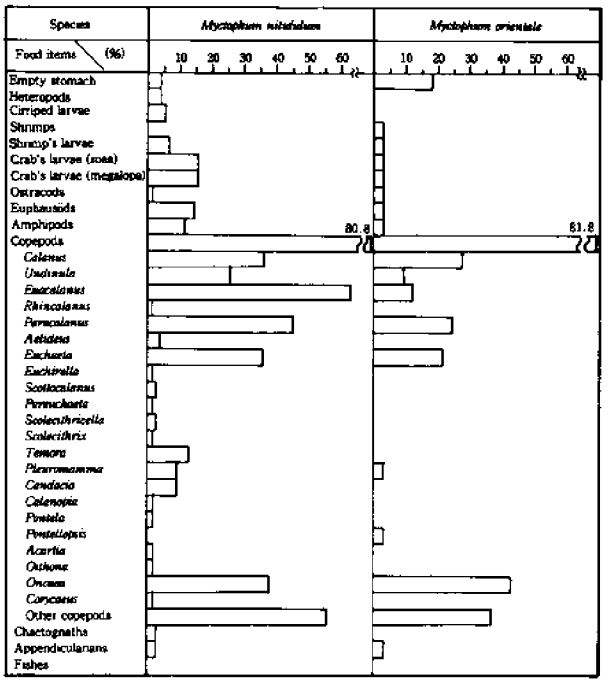

Figure 2. Percentage composition of stomach content of two epipelagic species of lanternfishes collected in Suruga Bay, Japan. (Percentage based upon the total number of species examined.)

Food of *Myctophum orientale*

Copepods were the most abundant prey, being found in 27 (81.8 percent) of the 33 stomachs. The order of abundance was *Oncaea*, *Calanus*, *Paracalanus*, *Euchaeta*, *Eucalanus* and *Undinula*. Larvae of shrimps and crabs, micronektonic shrimps, ostracods, euphausiids, appendicularians, and amphipods were also taken by the fish. Six of the 33 stomachs were empty (Figure 2). The average index was calculated as 0.89, which was lower than in *M. nitidulum*. The food intake of *M. orientale* was 0.0095 g wet weight per stomach on the average. That of *M. nitidulum* was 0.0107 g/stomach (Figure 3).

It is notable that the stomach contents of the epipelagic lanternfishes, *M. nitidulum* and *M. orientale*, were characterized by a higher percentage of copepods and a diverse species composition.

Food of *Diaphus suborbitalis*

Copepods were most abundant in the stomachs, being found in 407 (71.3 percent) of the

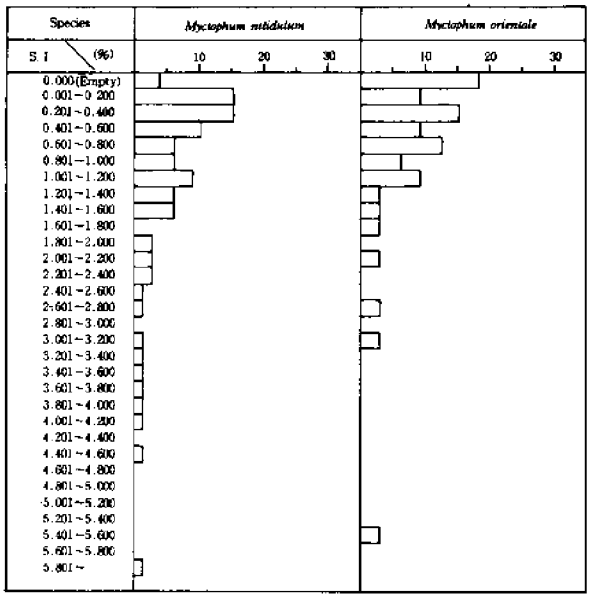

Figure 3. Percentage composition of stomach content index of two epipelagic species of lanternfishes in Suruga Bay, Japan.

571 fish. Among 22 genera of copepods, abundance was in the descending order of *Calanus*, *Oncaea*, *Eucalanus*, and *Euchaeta* (Figure 4). The stomach content index frequency distribution indicated that the class 0.201-0.400 was high (27.4 percent). Indices of the classes from 0.801 to 5.800 accounted for less than 5 percent of the total. The average index was 0.86. The weight of food per stomach was 0.0126 g on the average (Figure 5).

Copepods were most abundantly taken in the three seasons in 1976 and 1977. It was found that euphausiids were abundantly taken in the spring of 1977 (Figure 6). This resulted in a high frequency of high values of stomach content indices. Average seasonal stomach content indices were 0.3099, 0.7472, and 1.2297 in spring of 1976, fall of the same year and spring of 1977, respectively (Figure 7).

Food of *Benthosema pterota*

The most dominant organisms in the stomach contents were copepods, occurring in 246 (64.7 percent) of the 380 stomachs. Twenty-five of the 380 stomachs were empty. Copepods occurred in the descending order of *Oncaea*, *Calanus*, *Eucalanus*, *Euchaeta*, and *Candacia* (Figure 4). In the frequency distribution of the stomach content index, the class 0.001-0.200 was highest (36.3 percent). Indices of the classes from 0.601 to 5.800 accounted for less than 5 percent of the total. The average index was 0.74. The weight of food per stomach was 0.0078 g on average (Figure 5).

There was no great variation in species composition with seasons, but euphausiids were abundantly taken in the spring of 1976 and 1977; the percentage was particularly high in the latter year (Figure 8). In 1975 and 1976 stomach content indices higher than 1.800 were completely absent, but in the spring of 1977 a large number of fish had high stomach content indices. Average seasonal stomach content indices were 0.2159, 0.4374, 0.1884, and 2.3928 in the fall of 1975, spring and fall of 1976 and spring of 1977, respectively (Figure 9).

The average stomach content index and weight of food per stomach of *Diaphus suborbitalis* (0.86, 0.0126 g) was slightly higher than in *Benthosema pterota* (0.74, 0.0078 g).

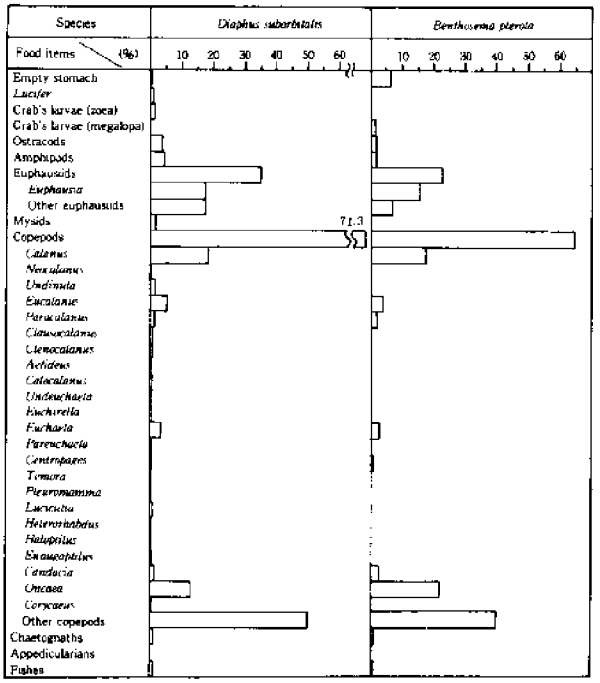

Figure 4. Percentage composition of stomach content of two mesopelagic species of lanternfishes collected in Suruga Bay, Japan. (Percentage based upon the total number of species examined.)

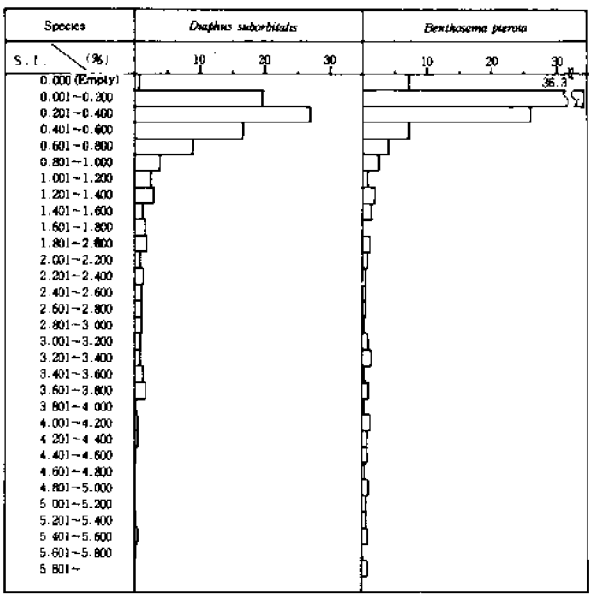

Figure 5. Percentage composition of stomach content index of two mesopelagic species of lanternfishes in Suruga Bay, Japan.

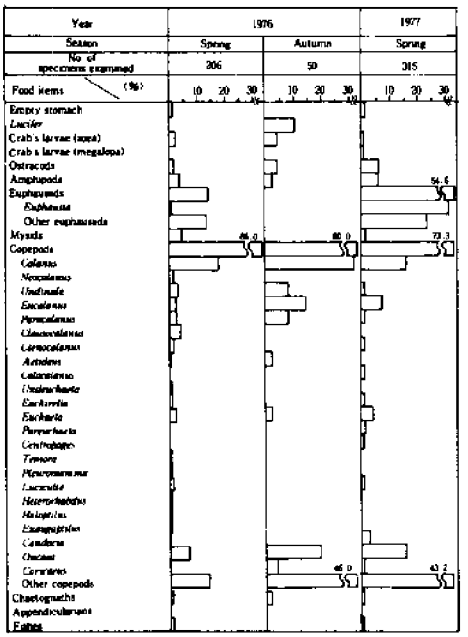

Figure 6. Percentage composition of stomach content of *Benthosema pterota* collected in Suruga Bay in 1975-1977.

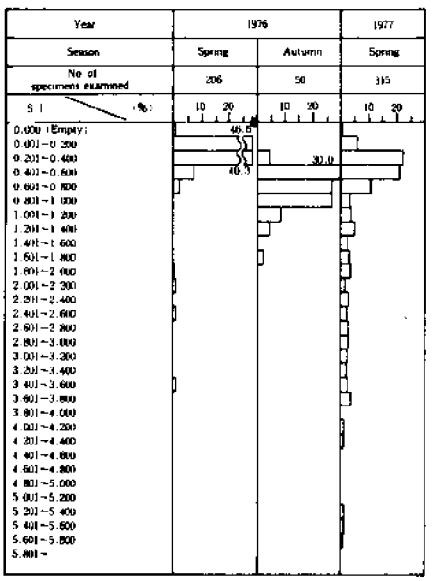

Figure 7. Percentage composition of stomach content index of *Benthosema pterota* collected in Suruga Bay in 1975-1977.

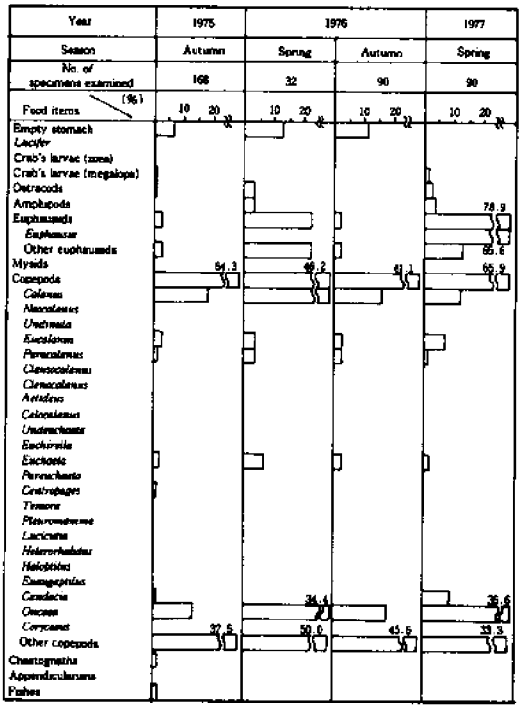

Figure 8. Percentage composition of stomach content of *Diaphus suborbitalis* collected in Suruga Bay in 1976-1977.

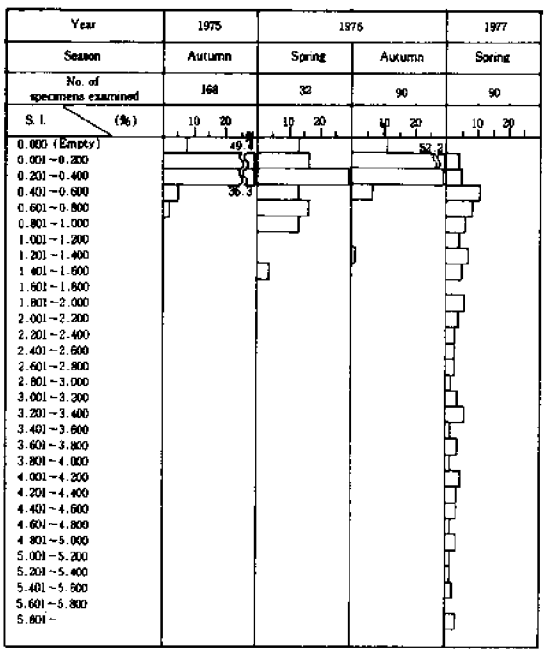

Figure 9. Percentage composition of stomach content index of *Diaphus suborbitalis* collected in Suruga Bay in 1976-1977.

Both mesopelagic species, *D. suborbitalis* and *B. pterota*, prey on similar numbers of copepods, but the species number of copepods was remarkably higher in *D. suborbitalis* than in *B. pterota*. The stomach content weight in these mesopelagic lanternfishes was generally lower than in epipelagic lanternfishes.

From their trophic relationships, lanternfishes in Suruga Bay are seen to be positioned between zooplankton, such as copepods, sergestid shrimp, euphausiids, etc., and fishes such as the lancefish, *Alepisaurus ferox*, the cutlassfish, *Trichiurus lepturus*, and the anglerfish, *Cryptosaras cousei*. Figure 10 illustrates the suggested pathways of the food web in the marine ecosystem of Suruga Bay (Kubota, 1973). In offshore areas skipjack, tuna, salmon, and marine mammals are known as voracious predators of lanternfishes. As there is no estimate of predation pressure of fishes at higher trophic levels on the lanternfishes, nor of feeding pressure of lanternfishes on zooplankton, the figure deals only in qualitative terms, but the important role of lanternfishes in the marine food web can be understood.

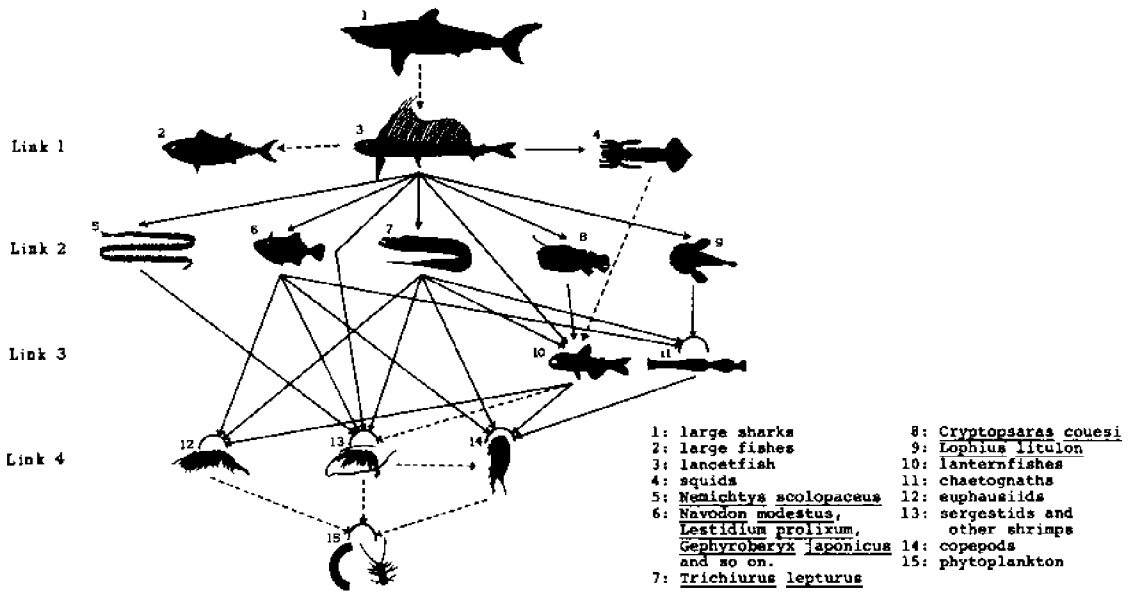

Figure 10. Diagram of suggested food web in Suruga Bay based on the stomach content analysis of lancetfish. (After Kubota, 1973).

A Note on Human Utilization of Lanternfishes

According to the fishery statistics compiled by FAO (1979) a species of lanternfish, *Lampanyctodes hectoris*, is landed by fishermen on the west coast of South Africa. Landings totaled 15,200 tons in 1972 and 42,400 tons in 1973, decreasing to 87 to 5,650 tons from 1974 to 1978. Ahlstrom, et al. (1976) reported that this fish contains fine quality oil.

In Suruga Bay, 18 species of lanternfishes are caught incidentally by seine nets in the sergestid fisheries (Kubota, 1980 MS). The dominant species are *Diaphus suborbitalis, Benthosema pterota, Diaphus garmani, Lampanyctus nobilis*, and *Diaphus watasei*. Although the lanternfishes are not sold on the market, local fishermen utilize large-sized species such as *Diaphus gigas, Diaphus sagamiensis*, and *Diaphus watasei* for food. After removal of the viscera, the fish are dipped in soy sauce and/or mirin, then preserved by drying. In the medium-sized species, *Diaphus suborbitalis*, the head is removed and the body is processed

into dumplings and prepared as fried fish, i.e., tempura or served as an ingredient in miso-soup. *Benthosema pterota* does not taste good and is not utilized. Species of *Ceratoscopelus*, *Lampanyctus*, and *Notoscopelus* have watery flesh and inferior taste.

Acknowledgments

I wish to express my sincere thanks to Dr. Sigeru Motoda of Tokai University, Shimizu for his kind help in the preparation of this manuscript. The author also thanks the crew members and the staff scientists of Tokaidaigaku Maru II for their helpful cooperation in collecting the samples. Many thanks are due to Mr. K. Hara, Mr. K. Sano, and other personnel of Yui Fishermen's Cooperatives, Yui Town, Shizuoka Prefecture, for their warm help in collecting samples sorted out from sergestid seine catches. The cooperation of Messrs. K. Nishida, S. Anjiki, M. Sugisaki, and K. Maruyama is also highly appreciated.

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Essential and Nonessential Amino Acids for Growth of Coho Fingerling (*Oncorhynchus kisutch*)

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Abstract

A feeding experiment was conducted with coho salmon (*Oncorhynchus kisutch*) fingerlings for six weeks to determine the essential amino acids for normal growth. An amino acid test diet was fed *ad libitum* three times a day to 55 fish stocked in individual tanks. Water quality and temperature were controlled by constant supply of well water (16.5°C) to each tank at the rate of 1.5 to 2.0 liter per minute. The fish fed diets deficient in each of arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine failed to grow until the deleted amino acid was added to the ration. The fish fed diets deficient in each of alanine, aspartic acid, cystine, glutamic acid, glycine, proline, serine, and tyrosine grew as well as those fed the complete diet. The essential amino acids for the growth of coho salmon fingerlings were the same ten amino acids reported to be essential for growth of other fishes.

Experiment

Experimental Fish

Coho salmon eggs were obtained in December 1977 from Fujinomiya Hatchery of Nichiro Gyogyo Co. Ltd. at Fujinomiya, Shizuoka Prefecture. After hatching, the fry were kept in tanks (50x100x50 cm) at 16.5°C on beef liver, commercial formulated feed, and a basal amino acid diet in that order. The body weight at the start of the feeding experiment was about 3 g.

Feeding Method

Test diet was fed *ad libitum* three times a day to 55 fish stocked in individual polyvinyl chloride tanks (20x50x30 cm). Water quality and temperature were controlled by constant supply of well water (16.5°C) to each tank at the rate of 1.5 to 2.0 liter per minute as well as by aeration. All fish were weighed biweekly after being anesthetized with MS 222 solution (1/10,000). The feeding experiment was conducted for six weeks. After the fifth week, recovery tests were conducted with the groups showing retarded growth by replacing the deficient diets with the complete basal diet.

Test Diet

The composition of the basal diet is shown in Table 1. All the amino acids used were in L-form. Lysine was in the hydrochloride form and others in the free form. The composition of amino acid mixture was simulated to that of the coho salmon eggs. Test diets were prepared by deleting a single amino acid from the basal diet and replacing it with a cellulose powder. The amino acid mixture level in the basal diet was fixed at 36.2 percent (30 percent

Table 1. Composition of amino acid test diet for coho salmon.

Amino acid mixture*	36.2%
Dextrin	16.0
Cellulose powder	23.3
Vitamin mixture	4.5
Mineral mixture	6.0
Mixed oil (corn 2: cod 1)	9.0
Carboxymethylcellulose	5.0

*L-Agr 2.0, L-His 1.0, L-Ile 1.9, L-Leu 3.1, L-Lys. HCl 3.7, L-Met 1.0, L-Phe 1.7, L-Thr 1.9, L-Trp 0.5, L-Val 2.3, L-Cys 0.6, L-Tyr 1.5, L-Ala 2.9, L-Asp 3.1, L-Glu 4.1, L-Gly 0.9, L-Pro 2.0 and L-Ser 2.0.

crude protein, N x 6.25) after a preliminary experiment. To adjust the pH value of the diet, a necessary quantity of 25 percent NaOH was added to the water before mixing with dry ingredients and the diet was adjusted to pH 6.5 to 7.0. The ingredients were well mixed and pelleted. Dry diets were used in this experiment.

Results and Discussion

The results of the feeding experiment are shown in Tables 2 and 3, and Figure 1.

Table 2. Results of feeding experiment for essential amino acid of coho salmon (4 weeks).

Deficient amino acid		Control	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val
Average body wt.(g)	Start	2.90	2.91	2.97	2.89	3.00	3.02	3.00	2.96	2.89	2.92	2.96
	Final	3.97	2.61	3.01	2.88	2.82	2.87	2.88	2.86	2.78	2.76	2.75
Daily growth rate (%/day)		1.12	-0.39	0.05	-0.01	-0.22	-0.18	-0.15	-0.12	-0.14	-0.20	-0.14
Feed efficiency (%)		42.0	—	3.3	—	—	—	—	—	—	—	—
Mortality (%)		1.8	0	0	0	1.8	0	0	0	0	0	0
Feeding rate (%/day)		2.62	1.71	1.50	1.46	1.73	1.36	1.46	1.47	1.33	1.39	1.48

Deficient amino acid		Cys	Tyr	Ala	Asp	Glu	Gly	Pro	Ser
Average body wt.(g)	Start	2.93	2.93	2.93	2.89	2.96	2.93	2.92	2.91
	Final	4.04	4.06	4.17	4.11	4.06	4.05	4.09	4.05
Daily growth rate (%/day)		1.15	1.16	1.26	1.26	1.13	1.16	1.20	1.18
Feed efficiency (%)		45.7	46.3	47.3	46.3	41.5	43.7	46.4	43.9
Mortality (%)		0	0	1.8	0	1.8	1.8	0	0
Feeding rate (%/day)		2.58	2.48	2.61	2.70	2.70	2.75	2.57	2.67

Table 3. Results of recovery test (2 weeks).

Deficient amino acid		Control	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val
Average body wt. (g)	Start	3.97	2.61	3.02	2.88	2.82	2.86	2.89	2.87	2.78	2.77	2.75
	Final	4.64	3.08	3.42	3.38	3.40	3.36	3.24	3.46	3.38	3.29	3.24
Daily growth rate (%/day)		1.11	1.18	0.89	1.14	1.34	1.15	0.82	1.34	1.40	1.23	1.17
Feed efficiency (%)		31.5	40.0	38.1	43.5	57.6	44.0	29.2	48.2	49.0	47.8	42.7
Mortality (%)		1.8	0	0	0	1.8	0	1.8	3.6	0	0	0
Feeding rate (%/day)		3.52	2.91	2.32	2.61	2.60	2.64	2.86	2.75	2.75	2.61	2.74

The fish on diets deficient in each of arginine, histidine, lysine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan and valine showed a loss of appetite in three to four days and retarded growth at the end of two weeks. Remarkable differences in the average body weight of these groups and that of the basal diet group were observed at the end of four weeks. All the groups of fish showing a retarded growth were given the basal diet for two weeks. In the recovery test, the fish recovered their normal appetite within two to

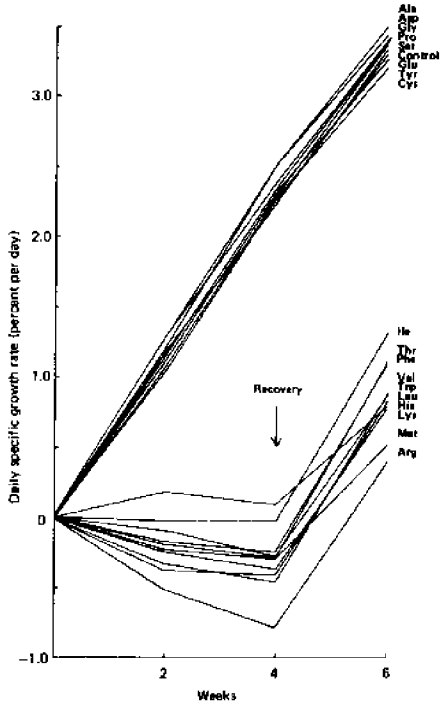

Figure 1. Growth curves of the experimental fish.

three days and showed a rapid recovery of growth.

These results clearly indicate that coho salmon fingerlings require ten amino acids: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine, as essential for normal growth. On the other hand, the fish receiving diets lacking each of alanine, aspartic acid, cystine, glutamic acid, glycine, proline, tyrosine, and serine showed a growth comparable to that of fish on the basal diet, indicating that these amino acids are nonessential.

By using an amino acid test diet, the amino acids essential for the growth of coho salmon fingerlings were clearly postulated to be the same as required by other salmonids (Halver, *et al.*, 1957; Halver and Shanks, 1960; Shanks, *et al.*, 1962), channel catfish (Dupree and Halver, 1970), eel (Arai, *et al.*, 1972), and carp (Nose, *et al.*, 1974).

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Section VIII
Advances in Shellfish Culture

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Aug. 1980, Anchorage, Alaska

Shellfish Aquaculture in the Pacific Northwest

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Introduction

Shellfish culture in the State of Washington began about a century ago when early settlers were encouraged to select intertidal lands for oyster culture. The Bush Act, enacted by the legislature, provided that such areas could be held as private land as long as they were used for oyster culture. Later, the Bush Act was amended to provide a clear title to such lands upon payment of a modest fee to the state. About 60 percent of the tidelands in Washington state bays were purchased by private individuals before the legislature prohibited this in 1974.

During the early days, Oregon and California permitted purchase of intertidal lands in coastal bays, although to a lesser extent than in Washington. Leasing of state tidelands and subtidal bottoms for culture of shellfish is still permitted in Washington, Oregon, California, and Alaska. In addition, the water surface and the water column are also leased by all four states for aquaculture.

The availability of space in the coastal zone had permitted large scale development of shellfish aquaculture in the Pacific coast states. In contrast, it is extremely difficult to obtain private control over tidelands on the U.S. Atlantic coast where state laws generally permit the public use of beaches to the high tide level. However, most eastern and Gulf of Mexico states lease the water column and subtidal bottoms for aquaculture.

Oyster culture began in Washington state with the harvesting of natural stocks of the native Olympia oyster (*Ostrea lurida*) in areas where natural setting provided adequate seed. Subsequently, the state established seed oyster reserves in certain areas where consistent setting occurred and permitted growers to transplant seed to private beds upon payment of a modest fee. This encouraged the expansion of oyster culture and led to the development of methods for increasing setting on private beds by providing substances such as clean oyster shells or concrete covered cardboard or wood to which the oyster larvae could attach. Aquaculture in the Pacific Northwest was also encouraged by the introduction of exotic species such as the eastern oyster (*Crassostrea virginica*), and the Japanese oyster (*Crassostrea gigas*).

Clam culture on the Pacific coast was also encouraged by increased demand and limited supply. For many years it was possible to purchase a license in the State of Washington to dig clams commercially on state owned tidelands. This practice was terminated about 15 years ago as most of the suitable tidelands were privately owned or leased from the state. A clam farm license is now required and this permits the culture and harvesting of clams on privately owned or leased lands. The State of Oregon still licenses commercial clam diggers,

although the areas available for harvesting are restricted and the clam populations are generally too low to support much beyond recreational digging.

In Alaska, there are large quantities of clams on public intertidal beaches; however, the commercial harvest of these stocks is severely limited as many areas are affected by the toxic dinoflagellate, *Gonyaulax*, which causes paralytic shellfish poisoning. As a result, the amount of natural clam stocks taken is extremely limited.

The following sections will discuss the status and potential expansion of oyster, clam, mussel, scallop, shrimp and freshwater crawfish aquaculture.

Oysters

Background

Oyster culture on the Pacific coast began with Washington's native or Olympia oyster (*Ostrea lurida*). This small oyster, which reaches a maximum size of 65 mm in diameter, requires about four years to reach commercial size. It is grown in diked areas where the beds are covered by water even at low tide, and are therefore protected against drastic seasonal temperature changes. This type of culture is very labor-intensive, resulting in current wholesale prices of $120 to $150 per gallon of shucked meat. Small quantities of Olympia oysters are still produced in the southern part of Puget Sound, Washington, for gourmet restaurants.

More than 50 years ago, the eastern oyster, *Crassostrea virginica*, was introduced into several areas of Washington, Oregon and California; however, sustaining populations were not established. Aquaculture of this species (primarily in California) currently consists of transplanting market-sized oysters shipped from the Atlantic coast, to local waters for short periods of time before marketing.

The Pacific oyster, *Crassostrea gigas*, was first introduced to the Pacific coast of the United States from Japan in the early 1930s. It adapted well and is now the principal marketed species on the west coast. For many years, the industry depended upon seed imported annually from Japan. In 1978, however, oyster setting in Japan was poor and local demand was great, leaving no seed available for export to the United States. Since that time the price of Japanese seed has increased greatly in response to the high demand and changes in the value of the yen and the U.S. dollar, thus making the cost of imported seed prohibitive.

Fortunately, oyster seed can be collected in Hood Canal and Willapa Bay, Washington and Pendrell Sound, British Columbia where setting occurs during warm summers. However, seed from natural setting is not adequate to support the industry's needs. As a result, commercial hatcheries have been built and are now successfully producing seed oysters at competitive prices.

Present Status

Culture methods: The high tidal range (7 feet to over 20 feet) on the Pacific coast exposes large areas of intertidal land at low tide. Many of these areas have suitable substrates for on-bottom oyster culture.

Off-bottom culture using rafts, racks, or stakes has been tried at various locations including southern Puget Sound, Washington and Humboldt Bay, California. In general, these culture methods have been rejected because they are more expensive than on-bottom culture. It is important for oyster growers to minimize production costs because of the low profit margin involved in Pacific oyster culture.

Off-bottom culture is still used in Yaquina Bay and Coos Bay, Oregon, and Tomales Bay, and Drakes Bay in California. Raft culture is used at Yaquina Bay because of the lack of suitable intertidal beds and the availability of protected estuarine waters. Stake culture techniques are used in the intertidal beds in Coos Bay, Oregon and Tomales Bay, California,

areas which are generally too soft for on-bottom culture. In this system, wooden stakes four to five feet long are inserted into the substrate at about three foot intervals. Several large oyster shells with attached spat are tied to each stake. The accelerated growth rate and excellent quality which leads to higher market demand tend to offset the additional labor cost of this method of culture.

In both Tomales and Drakes Bay, California, oyster shells with attached spat are suspended from long fences or racks four to five feet high located in the lower part of the intertidal zone. This method increases the growth rate and also protects the oysters from predatory bat rays (*Holorhinus californicus*). Although a relatively small portion of the west coast oyster production comes from off-bottom culture, these methods hold great promise for the future when suitable intertidal beds are fully utilized and when the price of oysters increases to a level which will permit increased production cost.

Procedure: Seed attached to oyster shells is obtained from Japan, natural reproduction in Washington or British Columbia, or from west coast hatcheries. The seed is generally planted on-bottom, sometimes directly on the growing beds and sometimes in gravel (nursery) areas for the first year and then transplanted to the growing beds. Individual seed oysters produced in hatcheries require growth in screen trays until large enough to be planted on-bottom.

Growth varies generally with the latitude ranging from less than two years to market size in California, two to four years in Oregon and southern Washington, and four to six years in northern Washington, Canada and Alaska. During the growing period the oysters require certain care including separation of clusters, thinning, and re-laying on "fattening" beds. In addition, it is necessary to control predators such as starfish and bat rays, in certain places.

Harvesting is accomplished by three general methods: 1) removal of oysters from intertidal beds at low tide by hand, 2) use of drag (bag) dredges at high tide, and 3) by use of hydraulic (escalator) dredges at high tide. Oysters grown in off-bottom culture systems are harvested by hand or by mechanical systems.

Location: Pacific oysters are grown in the four U.S. states and in British Columbia with highest production in Washington and lowest production in Alaska. The locations of commercial oyster beds in Washington, Oregon and California are shown in Figures 1, 2 and 3.

The areas now used for oyster culture are shown in Table 1.

Table 1. Tidelands in acres usable for oyster culture in Washington, Oregon and California.

State	Total Area	Area in Production
Washington	42,000	16,000
Oregon	4,000	1,350
California	17,150	2,120
TOTAL	63,150	19,470

Processing and marketing: About 90 to 95 percent of the Pacific oysters are shucked and marketed as a fresh or frozen product. The oysters are sorted into standard market categories and packed in various size containers as shown in Table 2.

Table 2. Pacific oysters, standard market categories.

Size	No/10 oz	No/pt.	No/½ gal.	No/#10	No/gal.
Large	4-5	6-8	26-32	36-48	51-64
Medium	5-8	8-12	32-48	48-72	64-96
Small	8-12	12-18	48-72	72-108	96-144
Extra Small	12-18	18-30	72-120	108-180	144-240
Yearling	18-23	30-38	120-150	180-228	240-300
Cocktail	23+	38+	150+	228+	300+

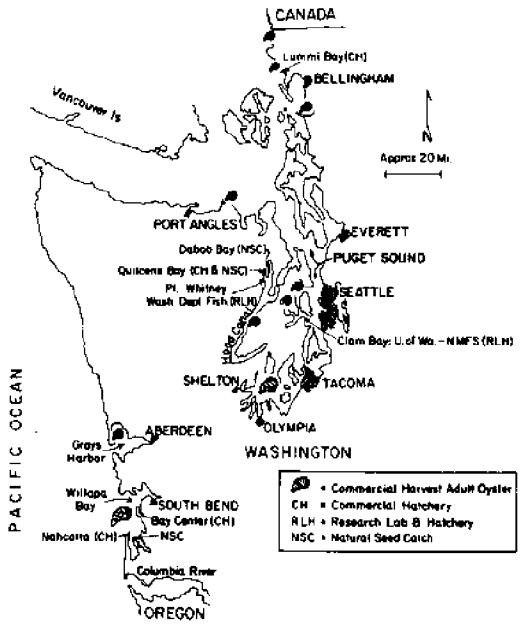

Figure 1. General location for commercial harvest of adult Pacific oysters, commercial hatcheries, research lab and hatcheries, and areas of natural seed catches for the State of Washington.

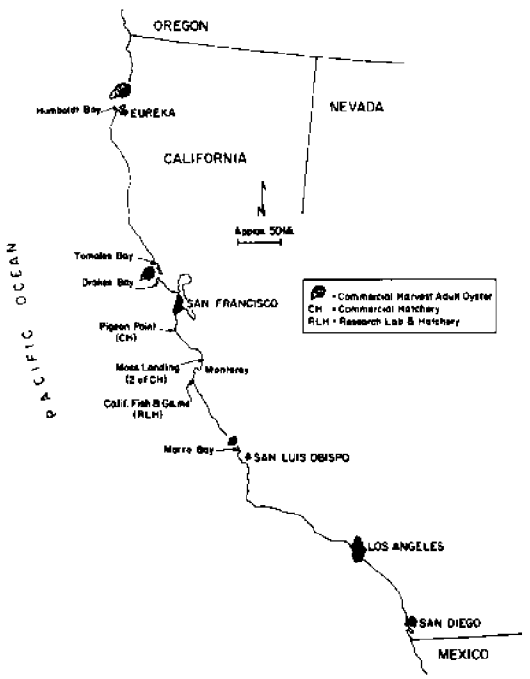

Figure 2. General location for commercial harvest of adult Pacific oysters, commercial hatchery and research hatchery for the State of Oregon.

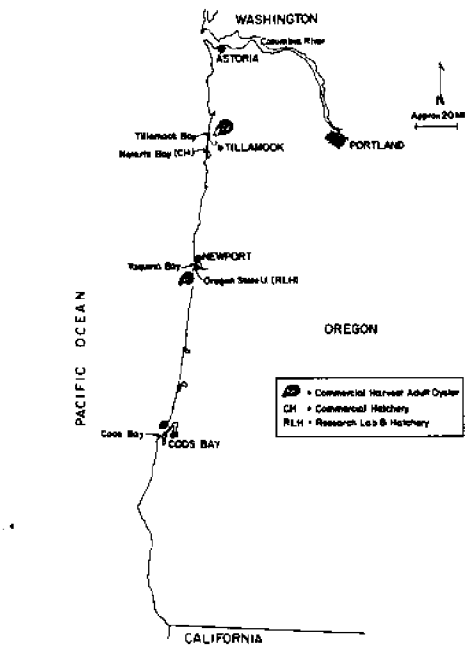

Figure 3. General location for commercial harvest of adult Pacific oysters, commercial hatcheries and research lab and hatchery.

About 5 to 10 percent of the Pacific oysters are marketed in the shell for shucking by the consumer or for the half-shell trade, but there is a growing acceptance of Pacific oysters as a half-shell product. Demand is especially high for oysters grown off-bottom or on a hard substrate as these stocks are reported to have a milder flavor than oysters raised on muddy bottoms.

Most of the oysters are channeled through major distribution centers in Seattle, Portland, San Francisco and Los Angeles. About 60 to 70 percent of the Pacific coast production are marketed in the Pacific coast states. Recent wholesale prices of shucked oysters in Seattle, Washington, are shown in Table 3.

Table 3. Wholesale prices, June 1980.*

Market Category	Container 8 oz	10 oz	Pint	Quart	Half Gal.	#10 can
Large					6.60	
Medium	1.03-1.04	1.22-1.25	1.90-1.97	3.80-3.93	7.90	11.10-11.85
Small	1.16	1.28-1.39	2.06-2.25	4.45-4.47	8.90	12.85-13.47
Extra Small	1.29	1.54-1.55	2.44-2.50	5.00-5.01	9.50-10.65	14.60-15.10
In shell = $0.13 each						

*National Marine Fisheries Service

Oyster prices have increased gradually along with inflationary trends but have yielded little additional return to the producer (see Table 4).

Production and trends: The United States is the largest oyster producing and consuming country in the world. The U.S. consumes 56 percent of the world's total annual production, or about 77 million pounds of oyster meat each year. Currently the U.S. produces about 50

Table 4. Wholesale price of shucked Pacific oysters, *Crassostrea gigas* in Seattle, Washington, cost index and oyster price adjusted to 1958 base.

Year	Oyster Price	Cost Index	Oyster Price Adjusted to 1958 Base
	Dollars/gal	Implicit price deflator	Dollars/gal
1958	4.10	99.97	4.10
1959	4.00	101.66	3.93
1960	4.10	103.29	3.97
1961	4.80	104.62	4.59
1962	4.75	105.78	4.49
1963	4.50	107.17	4.20
1964	4.50	108.85	4.13
1965	4.55	110.86	4.10
1966	5.75	113.95	5.05
1967	6.75	117.59	5.70
1968	6.75	122.31	5.52
1969	6.75	128.11	5.27
1970	6.75	134.86	5.00
1971	6.50- 7.50	142.11	4.57-5.28
1972	7.00- 8.00	148.00	4.73-5.41
1973	8.83- 9.98	156.58	5.64-6.37
1974	10.42-11.45	171.71	6.94-6.67
1975	10.20-11.60	188.23	5.42-6.16
1976	10.50-11.83	198.14	5.30-5.97
1977	12.40-14.50	207.97	5.96-6.97
1978	12.90-16.27	225.03	5.73-7.23
1979	14.80-18.80	247.37	5.98-7.48

million pounds of oysters per year and imports more than 20 million pounds. Although the per capita consumption of oysters in the United States has decreased during the past 20 years, the current demand is strong and an increasing trend of consumption appears probable.

Peak oyster production on the Pacific coast of more than 10 million pounds was reached in 1954 to 1956 (Figure 4). Since 1965, production has remained between 4 and 7 million pounds of meat. The decrease in production following the 1954 to 1956 peak was a result of increasing importation of lower priced canned oysters from Japan and Korea; U.S. canners could not compete with imported goods and therefore curtailed their production.

Problems

Seed supply: The development of the Pacific coast oyster industry was based on the availability of reasonably priced seed from Japan. Recently however, there have been setting failures and increased local demand in Japan, and changes in the value of the yen in relation to the dollar which have increased the cost of seed delivered to the United States by a factor of three. Since it is no longer economically feasible to import seed from Japan, growers depend upon hatcheries and natural reproduction. Natural reproduction occurs sporadically resulting in the need for expansion of hatchery production.

Predator control: The accidentally introduced Japanese oyster drill, *Ocenebra inornata* (formerly known as *Tritonalia japonica*) has been responsible for oyster stock losses of up to 90 percent in some areas. Some control is achieved by collecting the egg cases before hatching and by careful oystering practices. Starfish are moderately serious predators in some areas, but can usually be controlled by collecting them at low tide. The bat ray (*Holorhinus californicus*) which is a serious predator only in California, can be controlled by fencing or by utilizing off-bottom culture.

Paralytic shellfish poisoning: Blooms of the dinoflagellate, *Gonyaulax* (*Protogonyaulas*) *catanella*, occur periodically along the Pacific coast, usually during the summer. Oysters can

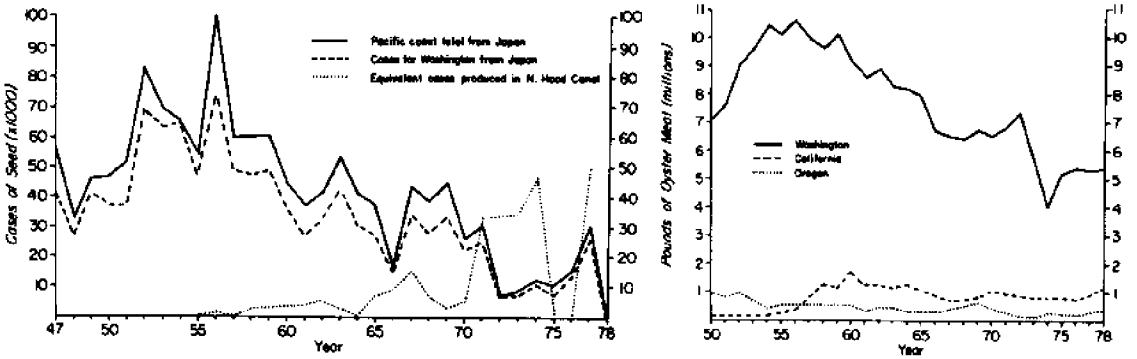

Figure 4. Production of Pacific oysters in Washington, California, and Oregon.

become toxic when blooms occur in oysters producing areas. State health agencies carefully monitor and close all harvesting in areas affected by the toxin. Historically, paralytic shellfish poisoning (PSP) occurred only along the ocean coast, entrances to coastal bays, and the Straits of Juan de Fuca. However, during the past three years *Gonyaulax* blooms have occurred in northern Puget Sound, resulting in closures of shellfish harvesting as far south as Seattle. PSP can adversely affect the overall shellfish market; however, the effect on the oyster market is low because oysters are generally marketed during the winter when PSP is absent or infrequent.

Markets: Since the production of Pacific oysters is entirely from private aquaculture, production is generally adjusted to meet the market demand. However, there are few if any industry-wide efforts being made to develop or expand the current market.

Water quality: The state health agencies certify areas as safe for shellfish harvesting according to federal guidelines. In general, west coast cities have upgraded their sewage treatment systems; therefore, it is unlikely that areas now used for oyster culture will become contaminated. Furthermore, current emphasis on water quality by the Environmental Protection Agency as well as state and local agencies has reduced the threat of increased industrial or domestic pollution.

Potential for Expansion of Oyster Culture

There is good potential for restoring west coast oyster production to the 10 million pound level of 1954 to 1956 through use of current culture techniques in unutilized areas. When adequate seed becomes available and when demand increases sufficiently, the application of off-bottom methods could easily expand production to more than 20 million pounds of meat per year.

Clams

Background

Abundant clam resources occur naturally along the coast of the United States, and clam harvesting provides employment for more fishermen and vessels than any other U.S. commercial fishery (Ritchie, 1977). More than 14 species of clams are commercially harvested in 18 states, however, the hardshell, softshell, surf, and ocean quahog account for 99 percent of

the volume and dockside value. The hardshell clam fishery includes the genus *Mercenaria* which composes 96.5 percent of all landings. Several other Pacific coast species are responsible for the remaining 3.5 percent. The surf clam, *Spisula solidissima*, has accounted for up to 69 percent of the volume and 25 percent of the dockside value of the U.S. clam landings in the past but production has decreased recently. The softshell clam, *Mya arenaria*, accounts for 12 percent of the volume and 20 percent of the dockside value. The ocean quahog, *Arctica islandica*, accounted for 1 percent of the dockside value in the past, with increased landings recently.

Clams are harvested largely from publicly owned intertidal or subtidal beds. Less than 10 percent of the U.S. supply results from private aquaculture. The following five Pacific coast species have potential for aquaculture:

1) The Pacific butter clam, *Saxidomus giganteus*.
2) The Pacific native littleneck clam, *Protothaca staminea*.
3) The Pacific Manila clam, *Tapes semidecussata (Venerupis japonica)*.
4) The Pacific geoduck, *Panopea generosa*.
5) The softshell clam, *Mya arenaria*.

Private clam culture has developed less rapidly than oyster culture because of the large supplies available from wild stocks, inability to efficiently collect seed from natural reproduction, lack of hatcheries (until recent years), and the high seed losses from predation. Even today there are few clam farms utilizing hatchery-reared seed clams and controlling predation. Clam farming procedures consist principally of periodic harvesting of those clams which reach commercial size. The total clam production from the State of Washington comes from privately controlled beds and is, therefore, considered a product of aquaculture; although a primitive form.

Present Status

Clam farming on the Pacific coast is either intertidal or subtidal. Native littleneck, butter, Manila, and softshell clams are harvested by hand digging from intertidal beds. Native littleneck and butter clams are also harvested from subtidal beds by hydraulic escalator harvestors. Geoducks and the associated horse or snow clams (*Tresus nuttalli* and *Tresus capax*) are harvested from subtidal beds by scuba divers using suction devices.

The shellfish teaching staff and students of the University of Washington, College of Fisheries have developed methods of protecting seed from predators until the clams reach a large enough size to avoid predators (Miller, et al., 1978). These methods are now being utilized on a commercial scale by the Squaxin Indian tribe in southern Puget Sound and by Webb's clam farm on San Juan Island. If these methods continue to be successful, clam culture (especially culture of the Manila clam) could be upgraded to the sophisticated levels of oyster culture.

Location: The highest level of clam culture on the U.S. Pacific coast occurs in the State of Washington where more than 60 percent of the intertidal land is privately owned. There are also small intertidal areas in Oregon and California, suitable for clam culture, which are privately owned or controlled.

Clam farming has not yet developed in Alaska because of the large quantities of unharvestable wild stocks and the problem of paralytic shellfish poisoning.

Processing and marketing: Most of the native littleneck and Manila clams, and about half of the softshell clams are marketed in the shell for use as "steamer" clams. Butter clams are usually shucked and sold fresh, frozen or canned for restaurant use or for retail sale. Some of the softshell clams are also shucked and sold fresh or frozen to restaurants. Both geoducks and horse (snow) clams are shucked, cut into steaks and frozen, mainly for export to Japan.

The price of clams varies with species and size. Native littleneck, Manila, and softshell clams marketed in shell will sell for $0.60 to $0.75 per pound at producer level and $1.25 to

$1.50 per pound at the retail level. Clams which require processing, such as the butter clam, bring as little as $0.15 per pound to the producer. Geoducks are priced at $0.50 per pound in the shell, $0.85 per pound minced, and $2.75 per pound as steaks at the producer level. Live softshell clams for steamers were priced at $38.00 per bushel in October 1979; breaded and frozen softshell clams sold for $2.75 per pound at the processor's plant.

Production and trends: The total U.S. clam aquaculture production in 1977 was 96 million pounds of meat representing about 29 percent of the world's landings. Aquaculture production in 1973 was 2.6 million pounds, but has increased to more than 7 million pounds because of the increased harvesting of subtidal clam stocks in Washington. In general the demand for clams is higher than landings of available wild stocks, providing a good opportunity for further development of clam aquaculture.

Problems

Seed: Only Manila clam seed is available from west coast hatcheries and quantities are limited.

Seed production: Experimental planting of 1.5 mm hatchery-produced seed has resulted in high losses from predation, waves or currents. Seed clams larger than 1 cm in length are able to burrow into the substrate, therefore avoiding predators and the effects of water movement. However, rearing seed clams of this size has proven too costly. Research at the College of Fisheries has shown that smaller, less expensive clams may be successfully utilized if they are covered with plastic mesh netting. The mesh netting is being tested in large-scale plantings. If successful, this method should provide a good basis for expanding clam culture.

Paralytic shellfish poisoning: Clams, like oysters, ingest the toxic dinoflagellate *Gonyaulax (Protogonyaulax) catenella* making them unsafe for human consumption. This is a major problem in Alaska and in coastal waters of Washington, Oregon, and California. State agencies monitor beaches for the presence of PSP. Closures in Washington primarily restrict the harvesting of clams during the summer at the entrance to coastal bays, in the Strait of Juan de Fuca, and recently in Puget Sound.

Water quality: Areas suitable for clam culture are certified by state agencies utilizing federal guidelines. Constant improvement of pollution controls along the west coast indicates a general trend toward improving water quality.

Potential for Expansion of Clam Culture

A good market exists for all clam species and prices are increasing. New farming techniques and the development of additional hatcheries should make it possible for clam culture to continue to expand, providing tidelands are available and the necessary permits can be obtained.

Mussels

Background

Mussels grow naturally in many areas throughout the world and are a major seafood item in many countries. The principal species in Europe and North America is the blue mussel, *Mytilus edulis*. Worldwide landings of this species are about 400 thousand metric tons in the shell. The major producers of mussels are: Spain, Netherlands, France, Denmark, Germany, the United Kingdom, and the United States.

Mussel culture began as early as the 13th century in Europe (Mason, 1971) and highly effective culture methods are used in many European countries.

Mussel culture in the United States is in its infancy. Researchers and private companies have engaged in mussel culture for about seven years and currently there are six commercial

mussel farms in the United States: four in Maine, one in Rhode Island and one in Washington.

Present Status

Methods: Raft culture methods in the United States are based on those used in Spain. Spanish production rates as high as 50 tons of meat per acre per year have been reported by Mason (1971). This is approximately 200 times greater than yields from any other type of husbandry in which animals are grown without supplementary feeding.

Raft culture consists of hanging ropes from floating platforms from early spring through the summer when seed mussels will set on the suspended ropes. In years of good settlement, the ropes will be covered with spat (seed mussels) by the end of July and continue to grow until the following spring at which time wooden dowels, about four inches in length, are pushed into the lay of the rope at 90° angles, at intervals of 12 to 18 inches. This prevents the heavy mussel clusters from sliding down the ropes (Lutz, 1974). By the next autumn, a large number of mussels will have reached marketable size (two inches or greater in length) and can be removed from the ropes. Mussels which are too small for marketing can be reattached to the ropes or placed in long narrow mesh tubes and returned to the water for further growth.

Locations: The blue mussel is widely distributed in the northern part of the United States and could be cultured in bays or estuaries where rafts could be anchored in protected waters. The surface area used for mussel culture is approximately 60 acres in Rhode Island, 14 acres in Maine and 5 acres in Washington.

Processing and marketing: In the United States, mussels are sold live in the shell. In Europe where production is extremely large, mussels are sold fresh, pickled, and more recently as frozen, cooked meats. Although the market for mussels in the United States has been very small, public interest has increased considerably. Mussels in the shell are being sold at retail for $1.00 to $2.00 per pound.

Production and trends: The total annual harvest from the six U.S. mussel farms approaches 125 metric tons (5,000 bushels) but production is expected to increase rapidly.

Problems

Demand: The market in the United States for mussels historically has been limited to ethnic groups from countries where mussels are an accepted food item. For many years small quantities of natural mussel stocks were harvested in New England and sent to New York where they sold at $0.50 to $1.00 per bushel.

On the Pacific coast the demand for mussels has been somewhat limited by paralytic shellfish poisoning problems. Recurring summer blooms along the coast of Washington, Oregon, and California have resulted in public apprehension concerning the product. More recently mussels have become a popular gourmet food item and demand is increasing.

Paralytic shellfish poisoning: Mussels, like oysters and clams, can become highly toxic after ingesting large quantities of *Gonyaulax (Protogonyaulax)*. Recent outbreaks of "red tide" in Puget Sound have stopped shellfish harvesting for extended periods of time during the summer and early autumn. Although mussels can be harvested safely during the winter, the negative publicity related to PSP outbreaks can adversely affect the market.

Predator control: Diving sea ducks, primarily scoters, find mussel farms extremely attractive. Large numbers of sea ducks have been reported to strip most of the smaller mussels (less than 25 mm in length) from culture ropes and tubes. Growers have used various sound devices to discourage the ducks but to date none has proven successful.

Seed: Although seed mussels can be obtained from natural reproduction in many areas, setting varies in intensity from year to year. In such cases it may be necessary to produce

seed mussels in hatcheries. Techniques for larval culture have been developed but have not been applied in commercial operations.

Potential for Expansion of Mussel Culture

There is much optimism at this time concerning the potential of mussel farming. There are no major problems which would prevent large-scale production; however, the extent of the market is unknown.

Scallops

Background

Atlantic coast sea scallop, *Placopecten magellanicus*, landings have ranged from 27 million pounds of meat in 1961 to 6 million pounds in 1973, and 31 million pounds of meat in 1978. The weathervane scallop, *Patinopecten caurinus*, occurs in moderate abundance along the Pacific coast from Alaska to Humboldt Bay, California. Annual harvests of weathervane scallops are less than 1 million pounds of meat.

The recent increase in prices has raised the question of the potential of scallops for commercial aquaculture. Research in Virginia has developed hatchery culture methods for the bay scallop, *Aequipecten irradians*, and commercial culture is planned for shallow bay areas of eastern Maryland and Virginia. If these ventures prove successful, bay scallop culture techniques could be applied as far north as Massachusetts.

Although larvae of the Atlantic sea scallop have been reared in the laboratory, there has been little interest in farming this species due to abundant stocks and lower market prices. In Japan, however, a similar scallop, *Patinopecten yessoensis*, has been raised commercially for a number of years. Methods have been developed for collecting naturally produced seed by placing used nets inside of onion bags and suspending these off-bottom in areas where scallops are abundant. The pelagic larvae attach to the strands of the net and grow to approximately 1 cm in diameter, then drop off the net into the bags, and are later removed and transplanted to suspended trays or cages for growth.

A substantial amount of research and development will be needed to provide an adequate scientific basis for sea scallop culture in the United States. It appears likely that some of the methods used in Japan could be applied to the culture of the weathervane scallop.

Present Status

There is no commercial aquaculture of sea scallops on the U.S. Pacific coast at the present time. The Washington State Department of Fisheries has recently received funding to develop culture techniques for the purple hinge scallop (*Hinnites giganteus*). Researchers in California have also been rearing this scallop under experimental conditions with prospects for commercial application.

Problems

Scallop culture in the Pacific northwest requires further investigation into appropriate culture techniques. Large-scale farming will require protected bays; farmers will need permits for anchoring rafts, or use of long line systems.

Potential for Development of Scallop Culture

There appears to be a high probability for developing successful scallop culture in northern Puget Sound where weathervane scallop stocks are now fished commercially. Although a commercial fishery does not now exist for the purple hinge scallop, it may also be a strong candidate for future commercial fishery efforts. However, this will require a period of ex-

tended research to be able to ascertain if commercial culture should be recommended. Expansion of weathervane scallop culture into Alaska, which supports a large-scale fishery, will require the development of culture systems suitable to the local environment, as well as additional knowledge concerning growth rates and survival.

Abalone

Background

Several species of abalone occur on the Pacific coast including the large red abalone, *Haliotis rufescens*, which occurs from Bodega Bay, California to Mexico and the pinto, British Columbia, or Japanese abalone, *Haliotis kamtschatkana*, which occurs from southern Alaska to Point Conception, California. This species is very similar to the Japanese abalone, *Haliotis discus hannai*.

A considerable amount of interest has developed in the aquaculture of abalone along the coast of California and Mexico. There is also the possibility of culturing abalone in the Pacific northwest by applying methods developed in Japan for culturing *H. discus hannai*.

In Japan, abalone are raised to a size of about 1.5 cm in prefectural hatcheries and sold to fishermen's cooperatives at about half the cost of rearing them. The members of the association plant the seed abalone along the coastline in areas where there is an abundance of kelp, and harvest them two or three years later when they reach market size.

Aquaculture schemes in California are based on a similar system; but with culture of seed in private hatcheries and rearing of juveniles on leased subtidal areas. None of these ventures has reached commercial viability at this time.

Present Status

Several companies in California including Monterey Abalone Farms, California Marine Associates, Pacific Ocean Farms, and Ab Lab have been developing culture techniques for the red abalone for nearly ten years. Monterey Abalone Farms is the most advanced and recently has announced an expansion which will permit commercial scale culture.

Some information from British Columbia based research is available concerning the growth, morphometry and breeding of the pinto abalone (Quayle, 1971). Recently the Washington State Department of Fisheries has begun to investigate the prospects of culturing abalone and will soon establish testing grow-out sites in northern Puget Sound.

Problems

Although there is a substantial body of knowledge available concerning the culture of abalone in California and Japan, a good scientific basis of culture information for the pinto abalone is lacking.

Potential for Development of Abalone Culture

Culture of the red abalone in California is nearing commercial viability and could become profitable in the near future due to the high consumer demand and market value.

There is a moderate potential for development of pinto abalone culture in Washington and perhaps Alaska; however, this will require an extended period of research, development of methodologies, and pilot scale testing.

Marine Shrimp

Background

The aquaculture of penaeid shrimp began in Japan when Dr. Fujinaga developed procedures for rearing the larvae of the kuruma prawn, *Penaeus japonicus*. Now there are more

than 20 shrimp farms in Japan with the total pond capacity of over 150 hectares. From these ponds more than 1,000 tons of shrimp are produced each year, selling at prices of up to $18.00 per pound.

Interest in marine shrimp aquaculture developed in the U.S. during the 1970s. Within the last ten years some research has been conducted to determine the feasibility of culturing the spot prawn (*Pandalus platyceros*) which occurs from Alaska to California. Spot prawns are large, with fine quality meat but are not as abundant as the smaller pink shrimp (*Pandalus borealis*) or the penaeid shrimps of the Gulf of Mexico.

The spot prawn has characteristics which indicate that it may be a suitable species for aquaculture. It is the fastest growing pandalid shrimp, although its growth rate is slower than that of many penaeids. It lives at salinities of 25 to 30 ppt and temperatures of 2 to 30°C and adapts well to shallow water environments. It is gregarious and there is no significant cannibalism, even in crowded conditions. Adult breeding stock can be captured at depths of 30 to 120 meters and transported to hatcheries with low mortalities. No serious disease problems have been reported to occur when these animals are in captivity.

Spot prawn larvae are easier to raise than penaeid shrimp larvae because of their abbreviated larval development, permitting them to feed on zooplankton immediately after hatching. During later development, the larvae and post-larvae have proven to adapt well to artificial diets. A research team at the National Marine Fisheries Service Aquaculture Experiment Station at Manchester, Washington succeeded in attempts to rear stocks to maturity in captivity. Survival of larvae to metamorphosis has routinely been 68 to 78 percent at 14°C in experiments.

Present Status

There have been no commercial spot prawn aquaculture attempts nor pilot studies to confirm the results of laboratory studies. Additional data will be needed to provide a sound scientific basis for the development of commercial spot prawn aquaculture. Studies regarding culture of the larvae of a similar species, *Pandalus hipponensis* in Japan by Dr. Yamamoto were reported at the North Pacific Aquaculture Symposium by Dr. Nobuo Tokumatsu.

Problems

The growth rate of the spot prawn is relatively low compared to tropical penaeid shrimp and it appears likely that culture to commercial size would require more than one year. It is known that larval development can be accelerated by raising the water temperature to 14 to 18°C. It also appears likely that this same procedure may apply to juveniles as well. Therefore, growing spot prawns in floating cages or in raceways which are supplied with warmer surface water should result in faster growth than experienced in nature.

Pilot studies are needed to verify the results of research conducted at Manchester and to provide a basis for analysis of the economic feasibility of production.

Potential for Aquaculture for Spot Prawns

It appears likely that commercial prawn farming could be developed in the Pacific northwest as a companion crop with salmon since both species require about the same environmental conditions. There is even a possibility of rearing the two species together since experiments at Manchester have shown that large prawns and small salmon can be kept in the same pen without predation losses. Being scavengers, the prawns would consume any dead salmon which dropped to the bottom of the pens thereby reducing feeding costs. A period of research, development and testing will be required before commercial aquaculture of spot prawns can be recommended.

Freshwater Crawfish

Background

Two groups of crawfish are cultured in the United States of the genera *Procambarus* and *Orconectes*. Both of these groups are grown in the southern United States with production of up to 45 million pounds per year.

The northern freshwater crawfish, *Pacifasticus leniusculus*, occurs naturally in lakes and rivers in the Pacific Northwest. The estimated annual fishery catch for the northern crawfish is approximately 500,000 pounds per year, much of which is exported to Europe. Crawfish is the most important freshwater fishery in Sweden and Finland with prices of $3.00 each in European restaurants; however high quality is required.

Present Status

The northern crawfish is not grown commercially in the United States but there is interest in the culture of this species in France, Sweden, Finland, Austria and West Germany. At the Second International Crawfish Symposium held in Louisiana in 1974, six countries reported culture of *P. leniusculus*.

Problems

The growth rate of the northern crawfish is much less than that of the species found in the southern part of the United States. It appears that culture to marketable size would take one to three years for Pacifasticus compared to one growing season for *Procambarus*.

The principal market for the northern crawfish is in northern Europe. Export would require air shipment from the Pacific Northwest to Europe.

Potential for Aquaculture of Crawfish

There is good potential for developing commercial crawfish aquaculture; however, an extended period of research and domestic market development will be required. After a sound scientific basis is developed, pilot scale tests will be needed to verify the commercial applicability of research results.

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Physiological Effects of 17β-Estradiol on the Japanese Oyster *Crassostrea gigas*

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Introduction

Unusual mass mortalities of oysters have occurred frequently in many parts of the world from the end of the 19th century to the present. We have carried out the physiological analyses of mass mortalities of oysters *Crassostrea gigas* in hanging cultures in Matsushima Bay, Miyagi Prefecture, Japan (Figure 1) (Imai, et al., 1965; Mori, et al., 1965a, b), and consequently the results have revealed that the artificial eutrophication in this bay induces excessive soft-body growth in the oysters and over-maturation of the gonad, possibly resulting in some physiological disorder and mass mortality (Mori, 1979).

In *C. gigas*, the presence of the Δ^5-3β- and 17β-hydroxysteroid dehydrogenase activities has been demonstrated histochemically in the limited tissues of maturing stage and a close association of the seasonal change in the latter enzyme activity with their sexual maturation has been confirmed (Mori, et al., 1964, 1965, 1966). In addition, our study has suggested the possible presence in the oyster of 17β-estradiol dehydrogenase which is specific for 17β-estradiol. These results, together with those on the glucose-6-phosphate dehydrogenase system (Mori, 1967), led us to assume that the biosynthesis of functional sex steroids such as 17β-estradiol exists in the marine bivalve.

Hence, the present paper deals with the various physiological effects of 17β-estradiol on the Japanese oyster *C. gigas* in relation to reproduction and energy metabolism. The ensembles of the results in this study will be discussed with special reference to the oyster mass mortality caused by eutrophication.

Effect of 17β-Estradiol on Sexual Maturation in Female *C. gigas*

Ovary Growth

The experiments were carried out in the maturing season from May to July. The oysters *C. gigas* cultivated for about two years in Onagawa Bay, Miyagi Prefecture (Figure 1) by the hanging method were used as the experimental materials. One group of these oysters was given injections of 17β-estradiol-3-benzoate (EB); the dosage was 100 μg/time/oyster in 0.1 ml aqueous suspension. The interval of injections was ten to 14 days. Injections were made into the gonad which had been exposed beforehand by cutting off a part of the right valve by means of a small hand-saw. The other group of oysters which did not receive injections were used as the control animals. The degree of ovary growth was estimated following the quantitative morphological analysis of Chalkley (1943), since the gonad of the oyster, unlike that of the scallop, is not easily separated from the body for determining the exact weight.

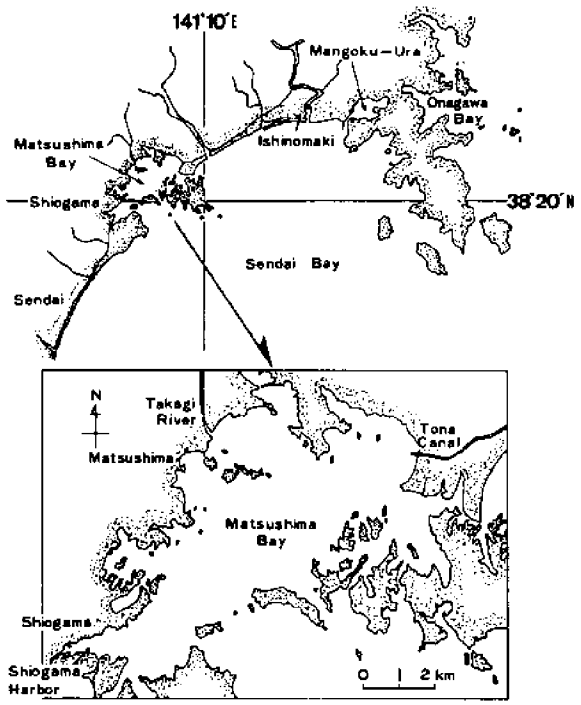

Figure 1. Map of Matsushima Bay and Onagawa Bay, Miyagi Prefecture, Japan, showing nearby cities.

Figure 2 shows the changes in the ratio of the total area of germ cells to the sum of the total areas of all morphologic tissue components in a section crossing the middle of the soft body of the female oyster after the administration of EB.

The area ratio of the steroid injected oysters was higher than that of the control animals in mid-July (total steroid injected, 400 μg/oyster), about 50 days after the start of injection. This result indicates that 17β-estradiol has an accelerating effect on the growth of ovary.

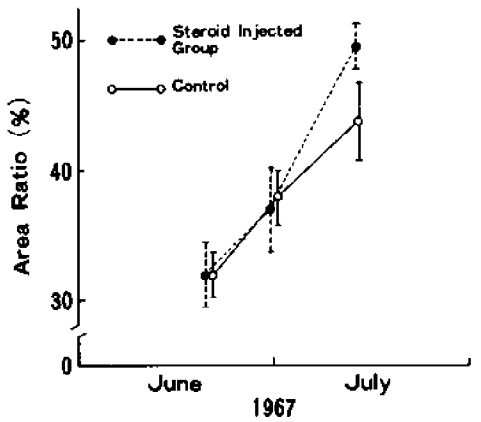

Figure 2. Changes in the ratio of the total area of germ cells to the sum of the total areas of all morphologic tissue components in a section crossing the middle part of the soft body of the female oyster after the administration of 17β-estradiol-3-benzoate (EB). Mean values (circles) and 95 percent confidence limits (vertical bars) are shown.

Egg Growth

Figure 3 represents the changes in the frequency distribution of the long-axis length of the egg nucleus of the same experimental oyster as that used in Figure 2. An accelerating effect on the egg growth was seen in mid-July.

From the results of Figures 2 and 3, it can be concluded that 17β-estradiol is capable of accelerating the sexual maturation in female C. *gigas*.

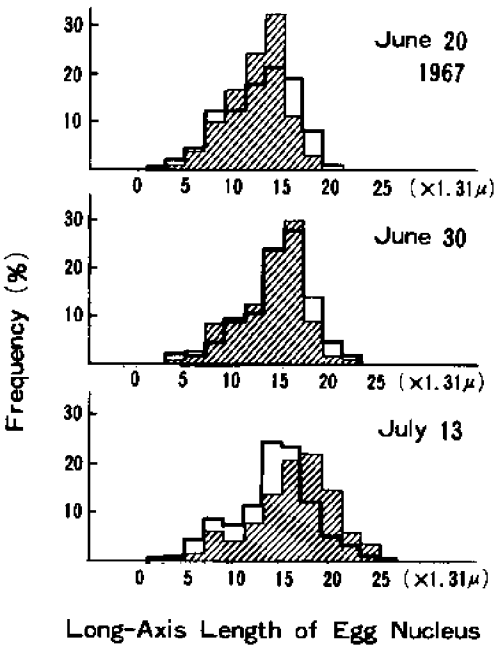

Figure 3. Changes in the frequency distribution of the long-axis length of the egg nucleus of oyster after the administration of EB. Shaded histogram: Steroid injected group. Unshaded histogram: Control.

Effect of 17β-Estradiol on Sex Reversal

Amemiya (1931) found that there was a close relationship between the rich nutrition and the occurrence of the high percentage of females in C. *gigas*. This nutrition theory is supported by the result of our investigation that the sex ratio (female/male) of cultivated oysters which grew rapidly was higher than that of natural oysters which were undergrown (Table 1). However, this does not thoroughly explain the mechanism of sex separation or sex reversal in C. *gigas* only by the nutrition theory.

Figure 4 shows the occurrence of 17β-hydroxysteroid dehydrogenase activity utilizing 17β-estradiol as a substrate in the nephridia of the same oysters as in Table 1. The order of strength of the enzyme activity in these oysters was unchanged throughout the stage of sexual maturation, i.e., $H > R_2 > R_1$. This is in accord with the order of good nutritive condition and that of high percentage of females in C. *gigas* (Table 1), suggesting that 17β-estradiol may have an effect on the sex separation of this bivalve. Hence, the next study was designed to obtain data concerning such an effect.

The methods of steroid administration to the experimental oysters in Onagawa Bay are summarized in Table 2. The study consists of three experiments with considerable difference in the respective times to start on injection into the connective tissue surrounding digestive diverticula. In experiments 1 and 2, in which EB injection started in March or April

Table 1. Sex ratio of two-year-old oysters from Onagawa Bay (12 July 1968).

Item	Oyster H	R_1	R_2
Total No. of oysters	115	102	100
♀	84	47	51
♂	31	55	49
☿	0	0	0
♀/♂	2.71	0.85	1.04

H: Oysters cultivated by the hanging method.
R_1: Natural oysters attached to intertidal rocks.
R_2: Natural oysters attached to rocks just under the ebb tide level.
Sex determination was made by the smearing method.

Table 2. Methods of steroid administration to the experimental oysters in Onagawa Bay.

Expt. No.	Period of expt.	Interval of injection (days)	EB injected μg/time/oyster	Total μg/oyster
1	12 March—21 May 1968	9—16	100	200—600
2	10 April—21 June 1968	5—12	20	40—160
3	26 May—13 July 1967	10—14	100	200—400

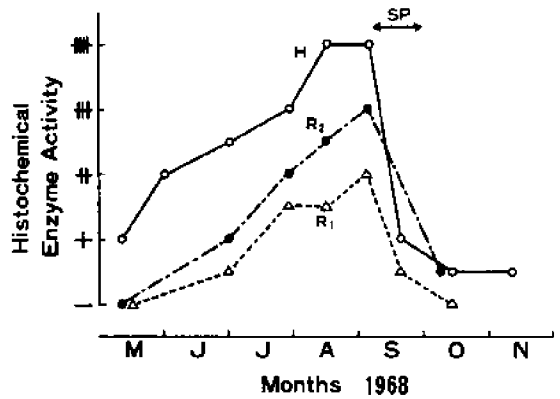

Figure 4. Difference in occurrence of 17β-hydroxysteroid dehydrogenase activity (substrate: 17β-estradiol) in the nephridium between cultivated oysters (H) and the natural oysters (R_1 and R_2) in Onagawa Bay. Abbreviations are the same as in Table 1. Bar (SP) shows spawning period.

when the sexual maturation of oyster was at the very early stage, the sex ratio (female/male) of S group was higher than that of C or N (Table 3). These results reveal the occurrence of sex reversal from male to female. In experiment 3, on the other hand, in which EB injection began late in May when the gonadal development of the oysters was just starting, the sex ratio of S was almost the same as that of N, indicating that no effect of EB on sex reversal was obtained.

The above data demonstrate that EB is capable of inducing sex reversal from male to female in *C. gigas* when EB injection starts at the very early stage of sexual maturation (in March or April). This fact, together with the previous one on the effect of EB on the female sexual maturation, indicates that 17β-estradiol functions as a female gonadal steroid in *C. gigas*.

Table 3. Effect of EB on the sex reversal in the oyster.

Item	Experiment No.								
	1			2			3		
	S	C	N	S	C	N	S	C	N
Total No. of oysters	36	13	10	44	20	20	44	—	83
♀	28	9	7	29	12	13	33	—	60
♂	6	4	3	8	5	6	11	—	22
⚥	2	0	0	7	3	1	0	—	1
♀/♂	4.67	2.25	2.33	3.63	2.40	2.17	3.00	—	2.73

S: Steroid injection.
C: Artificial seawater injection (control expt.).
N: No injection.

Effects of 17β-Estradiol and Testosterone on Respiration in Gonad

The effect of EB on the respiration in the tissues of maturing female oysters is shown in Table 4. Oxygen consumption was measured at 27°C using Warburg manometers, with single-arm vessels of about 17 ml capacity. An increase of about 32 percent (average) in the respiratory rate was observed in the ovary. A lower increase was found in the digestive diverticula, but no definite effect was obtained in the gill and pallial margin.

Table 5 represents the effect of EB on the tissue respiration of maturing male oysters. EB caused a marked increase of about 77 percent (average) in the spermary respiration. No effect was recognized in the digestive diverticula except one case, in which a considerable decrease was observed. Also no significant effect was seen in the gill and pallial margin.

The results of Tables 4 and 5 indicate that the oyster has a clear tissue specificity with the physiological effect of 17β-estradiol.

Testosterone, unlike 17β-estradiol, did not activate the respiration of oyster gonads and exhibited a slight decrease in it with the spermary (Table 6). This may be due to unsuitable dosage and/or improper experimental time.

Table 4. Effect of EB[1] on the tissue respiration of maturing female oyster.

Tissue	Experiment No.	Rate of respiration[2]		Increase in the rate of respiration (%)
		before tipping	30 minutes after tipping	
Ovary	1	22.8	30.6	34.2
	2	18.0	27.6	53.3
	3	25.6	32.0	25.0
	4	20.3	29.2	43.8
	5	20.0	29.0	45.0
	6	26.8	29.5	10.1
	7	26.2	30.1	14.9
	Average			32.3
Digestive divertic.	1	51.6	57.6	11.6
	2	34.3	42.9	25.1
	3	57.1	57.1	0
	4	40.3	52.3	29.8
	5	50.4	56.0	11.1
	6	48.1	52.2	8.5
	Average			14.4

[1] 100 μg of crystalline EB in aqueous suspension.
[2] μl O_2/100 mg fresh wt./hr. measured at 27°C.
No definite effect was obtained in the gill and pallial margin.

Table 5. Effect of EB[1] on the tissue respiration of maturing male oyster.

Tissue	Experiment No.	Rate of respiration[2] before tipping	Rate of respiration[2] 30 minutes after tipping	Increase in the rate of respiration (%)
Spermary	1	110.4	235.2	113.0
	2	122.4	187.2	52.9
	3	120.0	233.2	94.3
	4	114.4	174.0	52.1
	5	140.4	220.0	56.7
	6	104.8	200.0	90.8
	Average			76.6
Digestive divertic.	1	55.9	46.0	-17.7
	2	52.4	57.5	9.7
	3	39.0	37.7	-3.3
	4	43.8	45.0	2.7
	Average			-2.2

[1]100 μg of crystalline EB in aqueous suspension.
[2]μℓ O_2/100 mg fresh wt./hr. measured at 27°C.
No definite effect was obtained in the gill and pallial margin.

Table 6. Effect of testosterone[1] on the tissue respiration of maturing oyster.

Tissue	Experiment No.	Rate of respiration[2] before tipping	Rate of respiration[2] 30 minutes after tipping	Increase in the rate of respiration (%)
Spermary	1	201.3	180.4	-10.4
	2	121.2	114.8	-5.3
	3	186.3	161.8	-13.2
	Average			-9.6
Ovary	1	29.4	28.8	-2.0
	2	31.0	32.4	4.5
	3	25.5	26.4	3.5
	Average			2.0
Digestive divertic. of the male	1	50.4	46.8	-7.1
	2	36.0	37.2	3.3
	3	40.8	44.4	8.8
	Average			1.7
Digestive divertic. of the female	1	55.8	57.0	2.2
	2	58.2	53.4	-8.2
	3	45.6	40.8	-10.5
	Average			-5.5

[1]50 μg of crystals of testosterone propionate in aqueous suspension.
[2]μℓ O_2/100 mg fresh wt./hr. measured at 27°C.

According to Hathaway (1965), the conversion of 17β-estradiol to estrone by sperm preparations is more active in the oyster, *Crassostrea virginica*, than in sea urchins. When 17β-estradiol and testosterone are compared as substrates, sperm of *C. virginica* convert about six times as much estrogen as androgen. However, the physiological function of such metabolisms in spermatozoa remains to be investigated. In our experiment, the respiration in the spermaries of considerably mature oysters, *C. gigas*, was markedly activated by 17β-estradiol (Table 5). This finding, together with that of Hathaway, strongly suggests that

the metabolism of steroids by oyster sperm may be related to reproduction, and therefore the next investigation was attempted.

Effects of 17β-Estradiol on Fertilization and Development

The experiments were carried out in June using the oysters cultivated for about two years in Onagawa Bay by the raft-culture method, gonads of which were not yet fully developed. It was found afterward that spawning occurred in the middle of August. To obtain gametes, the animals were opened, and portions of the gonads were removed and shaken gently in seawater. After the eggs were washed by settling, the aqueous suspension for injection of EB was added to them. They were inseminated 5 to 10 minutes after the addition of the steroid. The supernatant fluid was replaced by new seawater 30 to 40 minutes after insemination.

The experimental results are given in Table 7. The rate of fertilization increased after the treatment of EB in each experiment. Particularly in the third experiment, the rate increased markedly from 35.0 to 94.4 percent by adding 3 μg/ml of EB.

Table 7. Effects of EB on the fertilization and development of maturing oyster.

Expt. No.[1]	EB added (μg/ml)	Hours after insemination	Temperature (°C)	Rate of fertilization (%)	Rate of development (%)[2]		
					B	G	B+G
1	0	4¼	25.0	82.5	10.2	0	10.2
	10	4¼	25.0	93.0	18.0	0	18.0
	20	4¼	25.0	94.5	50.0	0	50.0
2	0	5	22.0	80.6	7.1	2.4	9.5
	10	5	22.0	98.0	35.2	7.1	42.3
	20	5	22.0	100.0	25.5	11.9	37.4
	30	5	22.0	94.6	22.9	25.7	48.6
3	0	6¼	27.0	35.0	57.1	7.1	64.2
	3	6¼	27.0	94.4	17.6	52.9	70.5
	7	6¼	27.0	68.8	30.4	42.2	72.6
	10	6¼	27.0	64.3	44.4	22.3	66.7
4	0	6	27.5	62.6	13.9	8.0	21.9
	3	6	27.5	93.7	29.0	27.7	56.7
	10	6	27.5	97.1	28.2	26.3	54.5

[1]The materials are two-year-old oysters sampled in June in Onagawa Bay.
[2]B = (No. of blastula/No. of fertilized eggs) x 100
G = (No. of gastrula/No. of fertilized eggs) x 100
Salinity of seawater used for culture of fertilized eggs, 27.3 ppt.

This steroid also enhanced the rate of development in all experiments except the third. A five-fold increase in rate occurred in the first or second experiment. In the third, the rate of appearance of the gastrula became three to seven times higher by adding EB than that in the control, indicating that the steroid had the effect of increasing the rate of development also in this experiment.

These results seem to be closely related to the aforementioned fact that both female and male gonads are activated by 17β-estradiol, and to support the above suggestion that the metabolism of steroids by oyster sperm may be involved in reproduction.

Effect of 17β-Estradiol on Glycogenolysis in Female *C. gigas*

It has been well known that the glycogen content in oysters decreases remarkably with progressive development of the gonads (Figure 5). Figure 6 shows the seasonal variation in ciliary activity of the gills of the same animals as in Figure 5. It is found that the ciliary activi-

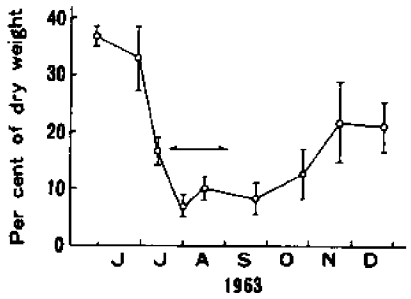

Figure 5. Seasonal changes in glycogen content of the whole soft body (except adductor muscle) of the Matsushima Bay oyster. Mean values (circles) and 95 percent confidence limits (vertical bars) are shown. Horizontal bar indicates spawning period.

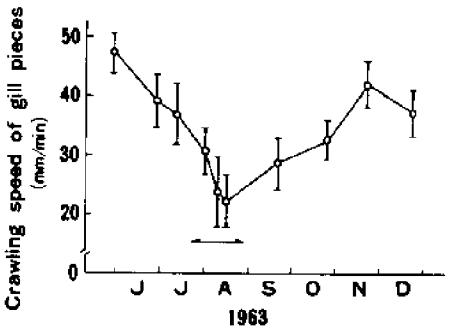

Figure 6. Seasonal variation in ciliary activity of the gill of the Matsushima Bay oyster. Mean values (circles) and 95 percent confidence limits (vertical bars) are shown. Horizontal bar indicates spawning period.

ty of the gill, which has been considered an adequate indicator for the physiological activity of the oyster, declines as sexual maturation proceeds and is minimal at spawning. The changes parallel the decreases in glycogen content of the whole soft body (Figure 5), revealing a close relation between physiological activity and glycogen content in the oyster. However, few data are now available on the metabolic causes and significance of the glycogenolysis.

Mori, et al. (1966) and Mori (1967) investigated the relationship between reproduction and steroid metabolism in *C. gigas*. The results of their studies evidenced a reciprocal relationship between seasonal variations in glycogen content and those in activities of 17β-hydroxysteroid dehydrogenase and glucose-6-phosphate dehydrogenase. Hence, it seems likely that at least a part of glycogenolysis during sexual maturation is related to the biosynthesis of sex steroids.

Experiments under Natural Conditions

Figure 7 outlines the experimental design for the effect of EB on the distribution in oyster tissues of PAS (periodic acid-Schiff)-positive substance (i.e., glycogen) which can be digested by saliva-treatment. In experiments 1 and 2, in which EB injection into the connective tissue surrounding digestive diverticula started at the very early stage of sexual maturation, no accelerating effect on glycogenolysis in the oysters was observed, although sex reversal from male to female was induced. In experiment 3, on the other hand, in which EB injection started late in May when the gonadal development was just beginning, the amount of

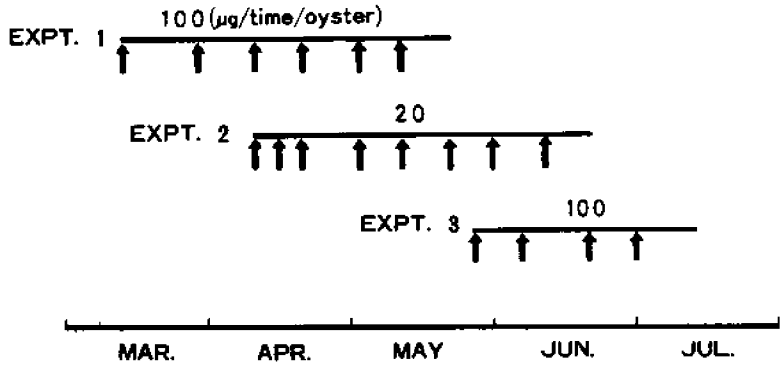

Figure 7. Experimental design for the effect of EB on the glycogenolysis of oysters under natural conditions in Onagawa Bay. Each of the arrows indicates the time when the steroid was injected.

glycogen in the interstitial tissue of gonad and the connective tissue of the mantle of the female oysters injected with EB was found to be lower than those of the control on 30 June (total EB injected, 300 μg/oyster) (Table 8), although no effect of EB on sex reversal was recognized. The same result was obtained in the interstitial tissue of gonad and the connective tissues around digestive diverticula and of the mantle of the female oysters sampled on 13 July (400 μg/oyster). These results indicate that 17β-estradiol has an accelerating effect on the glycogenolysis in the female *C. gigas*. The fact that such an effect is observed only in the female is of great interest in connection with the aforesaid evidence that 17β-estradiol functions as a female gonadal steroid in *C. gigas* (Figures 2 and 3, Table 3).

Table 8. Effect of EB on the distribution of glycogen in the oyster tissues (Expt. 3 under the natural condition).

		Date of Sampling					
		20 June		30 June		13 July	
Tissue	Sex	S	C	S	C	S	C
Interstitial	F	+++[15]	+++[10]	+[9]	+++[10]	±[10]	++[20]
tissue of gonad	M	+++[4]	+++[5]	++[5]	+++[5]	++[5]	++[5]
CT around	F	++[15]	++[10]	+[5]	++[10]	±[10]	++[20]
DD	M	++[4]	++[5]	++[4]	++[5]	++[5]	++[5]
CT around	F	+++[15]	+++[10]	++[5]	+++[10]	+[10]	++[20]
intestine	M	+++[4]	+++[5]	++[5]	+++[5]	++[5]	+++[5]
CT of	F	++++[15]	++++[10]	++[5]	++++[10]	+[10]	+++[20]
mantle	M	+++[4]	++++[5]	+++[5]	+++[5]	+++[5]	+++[5]

S: Steroid injection; C: Artificial seawater injection.
F: Female; M: Male.
DD: Digestive diverticula; CT: Connective tissue.
Figure shows the number of oysters examined.

Indoor Experiment

The experiment was performed in May, using the natural one-year-old oysters as the materials, which were reared in the apparatus consisting of the food-supplying, filter and culture vessels. The mean shell size of the materials was 4.8 cm in height, 3.3 cm in length and 2.1 cm in width. EB (0.01, 0.1, 1, 10, 100 or 1,000 μg/oyster/time) was administered as aqueous suspension to six apparatus every seven days from 3 to 24 May. Two other apparatus were employed for the control. Sampling of the experimental materials was conducted every seven days from 10 to 31 May. The histochemical method used for demonstration of glycogen is the same as that in Table 8.

As Table 9 shows, the glycogen amount of the groups of 0.01 to 0.04 μg (total EB ad-

Table 9. Effect of EB on the distribution of glycogen in the oyster tissues (Indoor experiment).

Tissue	Sex	EB applied (total μg/oyster)						
		0	0.01-0.04	0.1-0.4	1-4	10-40	100-400	1000-4000
Interstitial	F	++[15]	+[9]	+[7]	±[6]	+[8]	++[8]	±[5]
of gonad	M	++[24]	++[11]	++[13]	+[11]	+[10]	+[12]	±[9]
CT around	F	+[16]	±[9]	±[7]	-~±[6]	+[8]	+[6]	-[3]
DD	M	+[24]	+[11]	+[13]	+[11]	+[10]	±[12]	±[9]
CT around	F	++[16]	±[9]	±[7]	±[9]	++[8]	++[6]	-~±[5]
intestine	M	++[24]	++[11]	+[13]	+[11]	++[10]	++[12]	+[9]
CT of	F	++[16]	+[9]	+[7]	±[9]	++[8]	++[6]	±[3]
mantle	M	++[24]	++[11]	++[13]	++[11]	++[10]	++[12]	+[8]

F: Female; M: Male.
CT: Connective tissue.
DD: Digestive diverticula.
Figure shows the number of oysters examined.

ministered/oyster) and 0.1 to 0.4 μg was lower than that of 0 μg (control) in the connective tissue around the intestine of the female oysters. In the group of 1 to 4 μg also, the same result was obtained in the interstitial tissue of the gonad, the connective tissues around the digestive diverticula and intestine, and that of the mantle of the females. From these results, it is evident that 17β-estradiol has an accelerating effect on the glycogenolysis in the female *C. gigas*. However, no such effect was seen in the experimental groups of higher concentrations (10 μg and over/oyster) except the group of 1,000 to 4,000 μg/oyster. These facts indicate that the concentration of steroid administered is of special importance for appearance of such an effect. The decrease in glycogen amount which was observed in the 1,000 to 4,000 μg group should be considered to be qualitatively different from that occurring in the 4 μg and less group, because of the high frequency of mortality which is most likely due to serious pathological changes in the digestive diverticula.

In this indoor experiment, there were no indications of sex reversal, whereas the sexual maturation of the females of the 4 μg and less group was histologically observed to proceed normally.

The present study together with the previous one reveals that the acceleration of glycogenolysis by 17β-estradiol occurs in female *C. gigas* provided that sexual maturation proceeds normally without sex reversal.

Possibility for Regulations of Energy Metabolism by Way of Hormonal Control of Transhydrogenation

The above physiological results indicate not only that 17β-estradiol functions as a female gonadal steroid in *C. gigas*, but also that this steroid is involved in controlling the changes in glycogen metabolism during sexual maturation. Since glycogen is one of the main energy sources for the oyster, 17β-estradiol is assumed to be closely connected with the seasonal variations in the physiological activity. In Matsushima Bay, mass mortalities of oysters occurred during the spawning season, when such physiological factors as ciliary movement of the gill (Figure 6) and glycogen content of the whole soft body (Figure 5) attained their minima. Hence, it seems to be necessary to study the possibility for regulation of energy metabolism by 17β-estradiol in order to discuss the possible causes of the mass mortalities.

Recently, we found that 17β-hydroxysteroid dehydrogenase (substrate: 17β-estradiol) in *C. gigas* is able to utilize only NAD (but not NADP) efficiently as a coenzyme. This means that the so-called estrogen substrate theory advanced by Talalay and Williams-Ashman (1960) stating that estrogens act as coenzymes in the transhydrogenation reaction is not applicable to *C. gigas* (Mori, et al., unpublished). In sexually maturing *C. gigas*, 17β-estradiol injection

was observed to activate each of the 17β-hydroxysteroid, glucose-6-phosphate, NADP-specific isocitrate, and succinate dehydrogenases (Mori, et al., unpublished). We have arranged these data in the light of the estrogen-stimulable transhydrogenase theory proposed by Villee, et al. (1960). As a result, we have compiled a schematic diagram which illustrates the possibility for the regulation of energy metabolism by way of hormonal control of transhydrogenation in the oyster (Figure 8). However, their theory has not necessarily been entirely incorporated in this diagram, since we assume that the effect of 17β-estradiol in increasing the functional capacities of certain special tissues such as digestive diverticula and nephridia in *C. gigas* is not always mediated by the presence in those tissues of a specific estrogen-stimulable transhydrogenase. In our hypothesis (Figure 8), we emphasize the point that the 17β-estradiol dehydrogenase which specifically requires 17β-estradiol as substrate and NAD as coenzyme plays an important role in a transhydrogenation in the oyster.

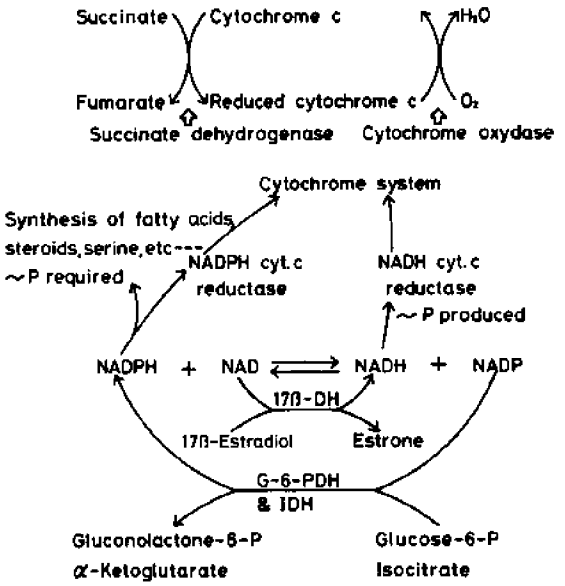

Figure 8. Diagram illustrating the possibility for the regulation of energy metabolism by way of hormonal control of a transhydrogenation in the oyster. 17β-DH: 17β-hydroxysteroid dehydrogenase; G-6-PDH: glucose-6-phosphate dehydrogenase; IDH: isocitrate dehydrogenase.

Matsushima Bay (Figure 1), one of the richest oyster-culture areas in Japan, is subject to heavy artificial eutrophication, mainly from the inflow of city and factory sewages. The physiological burden of *C. gigas* in hanging cultures in this embayment increases markedly with progressive development of the gonads; this increased burden coincides with eutrophication-induced accumulation of fatty material in the epithelia of the digestive organs; the oyster is thereby forced to depend on these accumulated fats for respiratory substrates in order to maintain its increased physiological needs. However, the fats are inefficient energy sources for the oyster, and therefore 50 percent mortality occurs during the spawning season. In addition to the conspicuous physiological changes stimulated by such steroids as 17β-estradiol, over-maturation of germ cells in the gonad occurs in the oysters of Matsushima Bay (Tamate, et al., 1965). This is accompanied by disturbance of the lipid and steroid metabolism (Mori, et al., 1965b, 1966). Over-maturation is a pathological phenomenon caused by a long residence of ripe oocytes or sperms in the gonad, and is characterized by an abnormal increase in glycogen and free fatty acids in the oocytes or the marked decline of succinate dehydrogenase activity and nuclear deoxyribonucleic acid polymerization in the sperms (Tamate, et al., 1965).

In connection with this over-maturation, it is of interest that only a very slight or no activity of 17β-hydroxysteroid dehydrogenase was found during late sexual maturation in the epithelia of the digestive diverticula of the oysters cultivated in Matsushima Bay (Mori, et al., 1966), indicating disturbance of the steroid metabolism. This is a noteworthy pathophysiological phenomena, since intense atrophy of the epithelium, namely, a strong enlargement of the lumen together with an inflammation has been histologically observed in most tubules of digestive diverticula of this oyster sampled in summer in the same bay (Tamate, et al., 1965).

Disturbance of lipid metabolism is characterized by the pathological symptoms indicative of fatty degeneration or necrosis in the digestive diverticula (Mori, 1979).

On the basis of the results and discussion described above, the author has compiled a schematic diagram illustrating the proposed effects of artificial eutrophication on the metabolism of *C. gigas* in relation to their physiological activity (Figure 9). It is reasonable to conclude that the seasonal mass mortalities of the oyster in Matsushima Bay are caused primarily by a physiological disorder, and a metabolic disturbance of the oyster as a result of intensive growth and maturation of the gonad under eutrophic conditions.

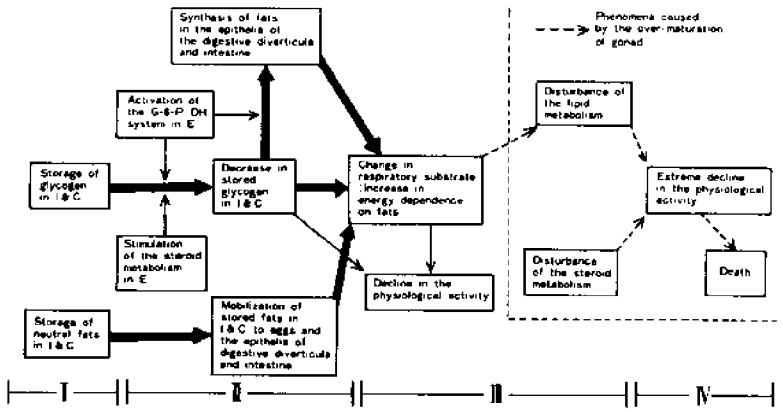

Figure 9. Schematic diagram illustrating the effects of artificial eutrophication on the metabolism of oysters in relation to their physiological activity. E: epithelia of nephridium, digestive diverticula and intestine; I&C: gonad interstitial tissue and connective tissues around the digestive diverticula and intestine; G-6-PDH: glucose-6-phosphate dehydrogenase. Abscissa shows the maturation stages — I: after spawning or before sexual maturation; II: during sexual maturation; III: immediately before spawning; IV: during spawning period.

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Seasonal Abundance of *Protogonyaulax* sp. Causing Paralytic Shellfish Poisoning in Funka Bay, Hokkaido, Japan, 1978-1980

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For the last ten to 15 years extensive endeavors have been made to culture scallop in Japan with the most promising results shown in the northern coastal areas. By artificial propagation, scallop production has reached more than 100,000 tons. The artificial propagation of scallop is usually carried out with the use of young shells which are collected by spat collector and cultured in cages for about three to six months. There are two culturing methods used: the sowing culture, and the hanging culture (Motoda, 1977).

Funka Bay (Volcano Bay) in Hokkaido is one of the most famous commercial scallop production areas where the hanging culture method is well established. This area alone produced about 60,000 tons in 1977. However, since 1977, mass mortality of scallops culture by the hanging method has occurred in Funka Bay. This mass mortality is similar to that which occurred in some areas of the northern coast of Honshu, the main island of Japan (Motoda, 1977).

In addition to the occurrence of a mass mortality, high levels of the paralytic shellfish toxin in scallop were discovered in 1978 in Funka Bay. Due to the high level of this toxin, the scallop fisheries in the bay suffered heavily; because the whole meat, including the digestive gland where most of the toxin is accumulated, was marketed and eaten in Japan.

Though Prakash, et al., (1971) reported that more than 400 persons died from paralytic shellfish poisoning in Japan, this number also included deaths caused by other kinds of shellfish poisoning. According to Noguchi and Hashimoto (1980), paralytic shellfish poisoning occurred five times and three persons have died as a result in Japan since 1948.

After a red tide of *Protogonyaulax* sp. occurred in Mie Prefecture (central part of Honshu), the presence of the paralytic shellfish poisoning was reported in 1975 (Hashimoto, et al., 1976). Monitoring of the toxin and survey on the causative organisms have been carried out in various areas in Japan, especially on the Sanriku coast, Mutsu Bay and Funka Bay, where scallop culturing is well established. In addition to paralytic shellfish toxin, the presence of a new type of shellfish toxin was recently reported from these areas by Yasumoto, et al., (1978), which has limited the marketing of scallops for several months each year.

Paralytic shellfish poisoning is caused by several species of the genus *Protogonyaulax* (Sommer, et al., 1937; and Prakash, et al., 1971). Investigations concerning the ecology of the causative organisms are not common, because of their infrequent occurrence. For the past three years, this writer has conducted investigations on the mechanism by which the scallop accumulates paralytic shellfish toxin in Funka Bay, Hokkaido. This paper describes the ecology of the causative organism and its relation to the toxification of scallops.

Locality and the Ocean Conditions

Funka Bay is located at the southwestern part of Hokkaido Island, and faces the Pacific Ocean. The diameter of this bay is about 60 km, and the depth is 100 m at the center. Scallop culturing areas extend along the coast. Periodic samplings of plankton and scallops were carried out at the station which is 1.5 km off Sawara and 68 m in depth. Supplemental surveys were carried out at the station off Shikabe and off Rebunge (Figure 1).

Oceanographic conditions of this bay were well investigated (Ohtani and Kido, 1980). The waters from the Tsugaru Warm Current (high salinity) begin to enter into the bay in late summer and remain through the winter. In early spring, the waters from the Chishima Cold Current (low salinity) begin to flow into the bay and remain until summer. Low water temperatures of about 2°C around February and a high water temperature of more than 20°C in summer were recorded in the surface layer (Figure 2). The thermocline is well developed in summer. Around the end of winter or early spring, nutrients of the water in the bay are consumed by a blooming of diatomaceous phytoplankton, which occurs around the end of winter or early spring (Nishihama, 1980), and remains at a low level in the euphotic zone through late autumn (Figure 3).

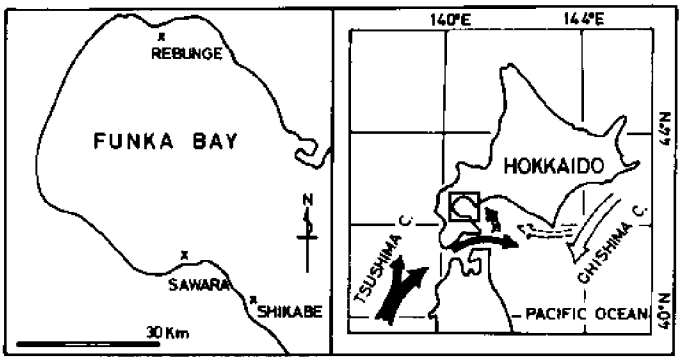

Figure 1. Maps showing sampling stations in Funka Bay (left side), and sea currents around Hokkaido Island (right side).

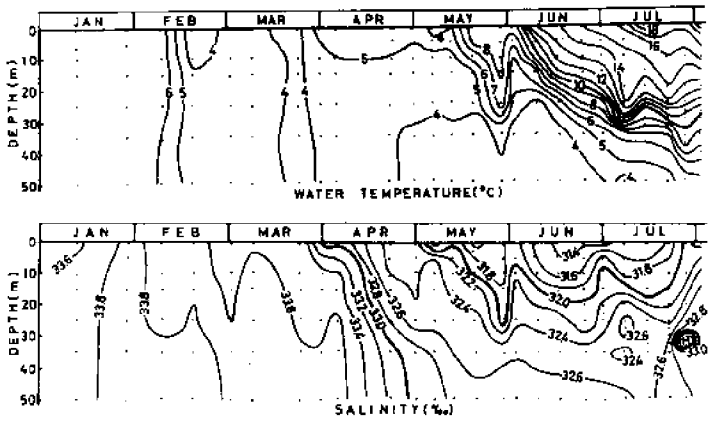

Figure 2. Seasonal change in water temperature and salinity off Shikabe, 1977-1979.

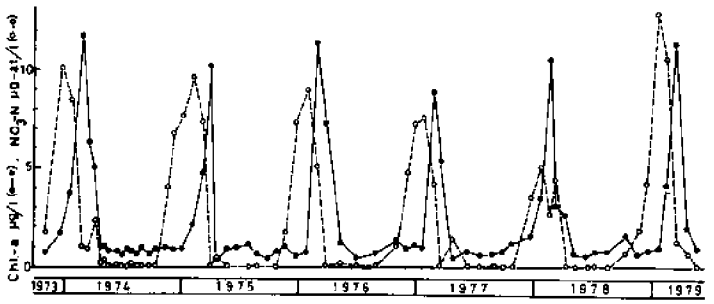

Figure 3. Seasonal change in levels of Nitrate-n and Chlorophyll-a in surface water (0-15 m depth) off Shikabe, 1973-1979. (Nishihama, 1980).

Materials and Methods

Routine seawater samplings were repeated at the station off Sawara in order to measure water temperature, salinity and phytoplankters. Seawater samples (1 liter each) were collected from various depths and were fixed and concentrated to 2 ml by repeated precipitation using various volumes of cylinders. A 0.1 ml of concentrated samples were used for counting *Protogonyaulax* cells (for details see Uchida, et al., 1980).

In order to inspect the toxicity levels of shellfish, two or three year old scallops (*Patinopecten yezoensis*), cultured by the sowing method, were hung in cages at selected depths. The digestive glands of scallops were examined for toxin by the mouse test (Horwitz, 1975). The toxicity levels were recorded as MU per 1 gram of the digestive gland.

Trend of Paralytic Shellfish Toxin at Funka Bay

Since 1976, monitoring the toxin of cultured scallops in Funka Bay has been continued periodically by members of the Hokkaido Institute of Public Health. The trend in paralytic shellfish toxin content of scallops at Funka Bay is shown in Figure 4. Low levels of toxin were observed in 1976 and 1977. In 1978, high levels of paralytic shellfish toxin were observed, and for the first time the marketing of cultured scallops from Funka Bay was limited to four months. In 1979, the highest concentration reached was 750 MU/g-digestive gland. Toxin levels increased around June in 1976, 1977 and 1978, and around April in 1979 and 1980.

In June 1978, the author started to conduct investigations on the causative organism.

The Causative Organism: *Protogonyaulax* sp. (GCF)

In this paper, a species belonging to the genus *Protogonyaulax* (*Gonyaulax catenella*) observed at Funka Bay in 1978 to 1980 will be abbreviated to "GCF". The account of the organism was given in Nishimama, et al., (1979).

The causative organism of paralytic shellfish poisoning is due to several species of Dinophyceae known as "*Gonyaulax catenella*" or "*Gonyaulax tamarensis*". According to Taylor (1979), they are now classified under the genus *Protogonyaulax*.

The initial investigation was aimed at searching for cells of the genus *Protogonyaulax* in Funka Bay at the time when high levels of the toxin were observed. A *Protogonyaulax catenella*-like species was found. This organism resembles *P. catenella*, however it does not form a long chain. Cultured cells of the organism are round in shape, similar to those of *P. tamarensis*. The organisms in the sea show intermediate characteristics between *P. catenella*

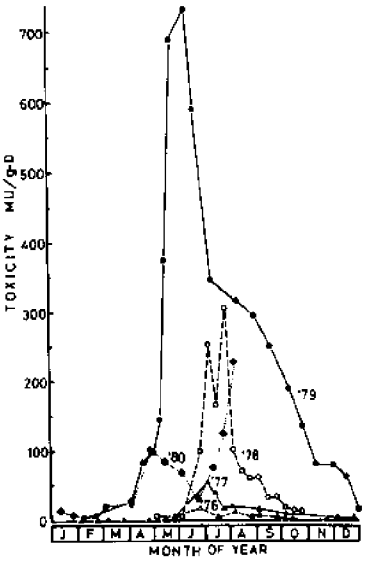

Figure 4. Trend in paralytic shellfish poison in the digestive gland of scallops at Funka Bay, 1976-1980.

and *P. tamarensis*. Therefore, they are considered as a *Protogonyaulax* sp. at the present time, and are referred to as "GCF" in this paper.

GCF presented a characteristic vertical distribution as shown in Figure 5. This data was obtained on 8 August 1978 at the station off Rebunge in Funka Bay (Uchida, et al., 1980). GCF cells were distributed only in the middle layer, where this organism was the dominant species. Though the temperature of the sea surface was 23°C, it suddenly went down with depth, because of a thermocline. At the 20 m depth it was 8°C, where GCF cells were aggregated.

Several taxa of the genus *Protogonyaulax* are toxic and others are non-toxic (Taylor, 1979). GCF was simply a member belonging to the genus *Protogonyaulax*. Therefore, the author tried to ascertain whether GCF made scallops toxic, using their characteristic vertical distribution.

Scallops with a toxicity score of 80 MU/g-digestive gland were hung at five different depths. At the beginning of the experiment, GCF cells were distributed in the middle layer as shown in Figure 6. After six days, they showed similar vertical distribution. Therefore, the scallops in the upper two cages did not take GCF cells. On the contrary, the scallops in the lower three cages took the cells as food. The toxicity was reduced in the upper two groups, but increased in those of the lower three, as shown in Figure 6.

Since the increase of the toxicity of scallops is associated with the presence or absence of GCF cells, it is reasonable to assume that the GCF is the causative organism.

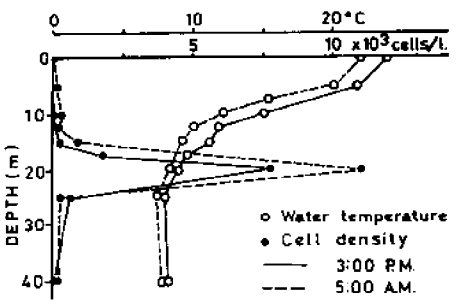

Figure 5. Vertical distribution of *Protogonyaulax* sp. off Rebunge in Funka Bay, August 1978. (Uchida, et al., 1980).

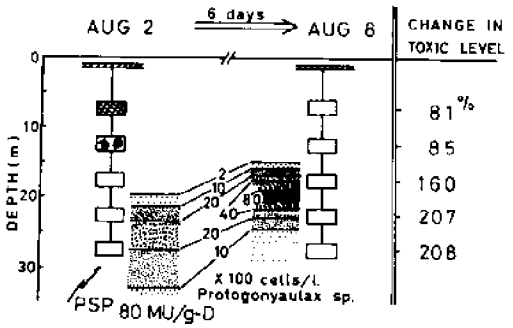

Figure 6. Experiment on the rate of toxicity change in scallop at various depths in Funka Bay, 1978. (Nishihama, et al., 1979).

In addition to this experiment in the sea, cultured cells of GCF showed that 7,000 cells contained 1 MU of the toxin. It is concluded that GCF (*Protogonyaulax* sp.) is the cause of the toxin in scallops in Funka Bay.

Seasonal Abundance and Vertical Distribution of *Protogonyaulax* sp. and Its Relation to the Toxification of Scallops

1978

The vertical distribution of GCF (*Protogonyaulax* sp.) and its relation to the water temperature condition from July to September 1978 were reported (Uchida, et al., 1980). In August, a thermocline was well developed, but it disappeared in September due to the inflow of warm water (Figure 7). At the same station, GCF appeared in the middle layer. The maximum density was 20,000 cells/liter. In September, GCF cells disappeared (Figure 8). The portion where the density is more than 1,000 cells/liter is presented by the dotted area. The depth where GCF cells aggregated fluctuated day by day. The three curves in the figure show water temperature. The high density zone of GCF was associated with the zone of 8° to 12°C. Water temperature condition for the growth of GCF was 8° to 12°C in its natural environment in the bay.

GCF cells were not observed in the surface layer in summer, where water temperature was more than 15°C. Since GCF need light for their growth, they ought to aggregate near the sea surface within the appropriate water temperature range. This is the reason why GCF cells occurred in the middle layer in summer. Therefore, a marked development of the thermocline near the surface in summer may be required for the propagation of GCF.

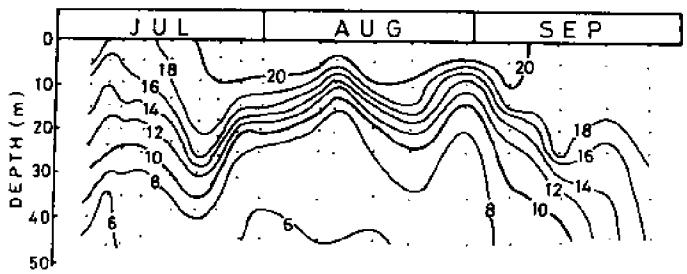

Figure 7. Changes in water temperature (°C) off Sawara in 1978. (Uchida, et al., 1980).

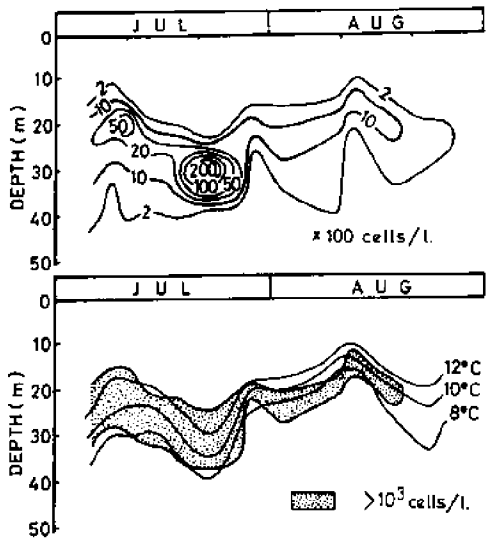

Figure 8. Vertical distribution of *Protogonyaulax* sp. and its relation to water temperature off Sawara in 1978. (Uchida, et al., 1980).

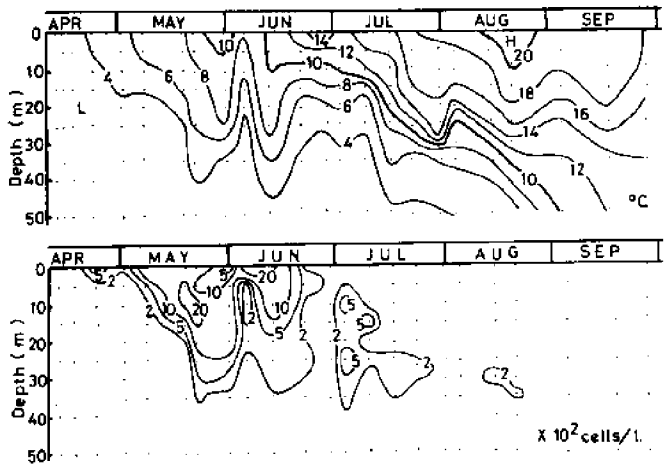

Figure 9. Change in water temperature (°C) and vertical distribution of *Protogonyaulax* sp. off Sawara between April and September, 1979. (Nishihama, et al., 1980).

1979

In the following year, 1979, the relation between the density of GCF cells and the toxicity of scallops was investigated (Nishihama, et al., 1980). A change in water temperature conditions from April to September is shown in Figure 9. The water temperature began to rise in May. From May to the middle of June, the surface water temperature was 8° to 12°C, and then it rose to more than 12°C. In July and August of 1979, thermocline was not formed as clearly as was observed in 1978.

In 1979, a very low density of the GCF cells was observed in March. Until the middle of April, however, they did not propagate actively because the water temperature was still very low. GCF began to propagate actively in the surface water in the middle of April with the

density of the cells reaching a maximum from the end of May to the beginning of June. After the surface water temperature rose to 14°C by the end of June, GCF disappeared in surface water, and appeared in the middle layer. Then, the density of the cells gradually reduced and disappeared by the end of August.

The displacement of GCF cells from the surface to the middle layer corresponded to the change of the depth where water temperature was about 10°C. The low density of the cells in the middle layer from July to August, 1979, is thought to be due to the less-developed thermocline.

In order to investigate the relation between the density of GCF cells and the toxin levels in scallops, scallops were suspended in cases at depths of 10 and 25 m. The toxicity of the scallops at 10 m depth suddenly increased at the end of May, and then began to decrease suddenly at the beginning of June. On the other hand, the toxicity of 25 m depth reached the maximum levels at the end of June (Figure 10). The change of toxicity at both depths corresponded very well with seasonal and vertical abundance of GCF cells. Toxicity levels of scallops at both depths continued to decrease gradually after September and almost disappeared by the end of January (Figure 11). It is interesting to note that due to the disappearance of the toxic plankton in the sea the toxicity of the digestive gland of scallops also suddenly decreased followed by a more gradual reduction.

1980

The toxicity level of scallops while low in April, 1979 (Figure 10), already exceeded the

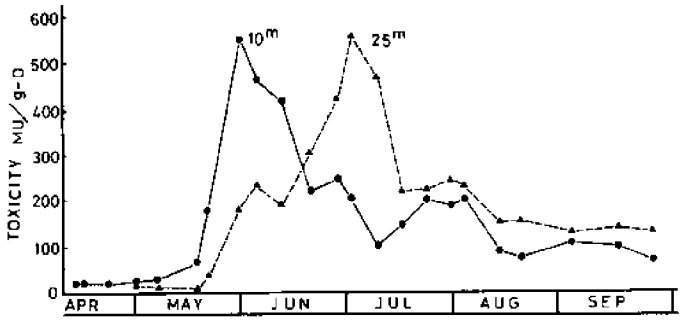

Figure 10. Change in the toxicity scores of scallops suspended at 10 and 25 m depth, off Sawara between April and September, 1979. (Nishihama, et al., 1980).

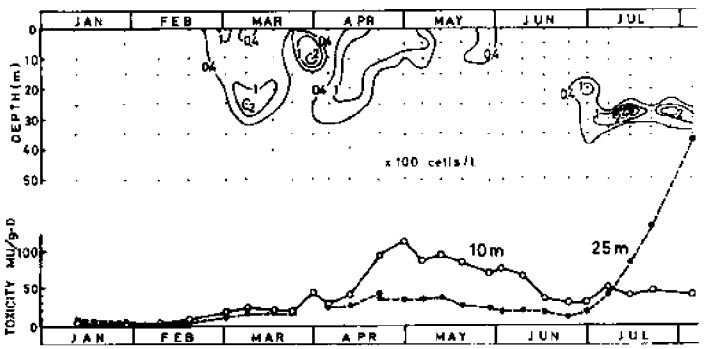

Figure 11. Vertical distribution of *Protogonyaulax* sp. and toxicity level of scallop off Sawara in 1980.

limit for marketing of scallops. Therefore, it was necessary to investigate the ecology of the causative organisms in early spring and to forecast the time when scallop toxicity would exceed the limit for marketing.

In 1980, a low density of GCF cells appeared in March. This was similar to the previous year's observation. In March, the toxicity of scallops increased gradually in spite of the very low density of GCF cells (Figure 11). An effective forecast based on plankton survey alone is very difficult, because accumulation of the toxin by scallops still goes on in spite of the very low density level of the causative organism, and because the increase of the toxicity is associated simultaneously with the increase of the toxic plankton.

GCF cells propagated to a density of several hundred cells/liter at the end of April. Then they disappeared for a short period in almost all depths of the water. At the beginning of July, they appeared again in the middle layer (the water temperature of the surface layer was already more than 15°C). The toxicity of scallops at 25 m depth rose again, too.

In 1979, GCF cells which appeared in low density in March and April, propagated quickly when water temperature rose to about 8° to 10°C at the end of May. But, in May and June 1980, GCF cells disappeared for a short period. In 1980, salinity dropped suddenly in April (Figure 12). This represents the inflow of the Chishima Cold Current which started to enter Funka Bay in April 1980. This exchange of seawater washed out the material for the propagation of GCF cells for a short period. Compared with 1979, the Chishima Cold Current started to enter the bay in February to March. This difference in the timing of the inflow of the Chishima Current explains why there were no GCF cells found in May and June 1980. Therefore, the stability of the seawater of the bay from spring to summer is thought to be a necessary condition for the propagation of GCF cells.

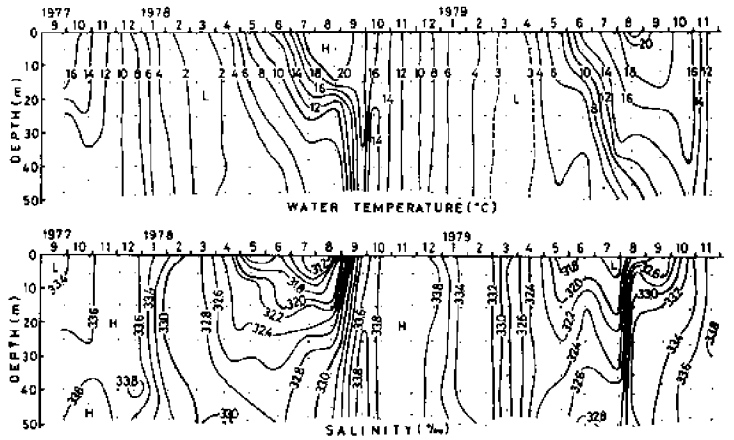

Figure 12. Change in water temperature and salinity off Sawara in 1980.

Conclusion

Protogonyaulax sp. is the cause of the toxin found in scallops in Funka Bay. They showed three different aspects on the seasonal abundance and the vertical distribution each year from 1978 to 1980. These three different types of occurrence of the organisms are explained by the fact that the optimum water temperature condition for the propagation of the species is approximately 10°C in Funka Bay. However, *Protogonyaulax* cells were not found in late autumn, when water temperature was about 10°C. Seasonal abundance and density level of the species are thought to be dependent on the degree of the development of a thermocline in summer, and on the degree of the stability of seawater conditions from spring to summer.

The change of the toxicity of scallops corresponded very well with seasonal and vertical abundance of *Protogonyaulax* cells.

Acknowledgements

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Rearing of Larvae of Deep-sea Macruran Decapod, *Pandalus nipponensis* Yokoya)

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Introduction

According to the taxonomical and faunistic studies by Yokoya (1933 and 1934) and the ecological studies on larvae by Kurata (1955 and 1964), five species belonging to genus *Pandalus* inhabit the coast of Japan and its environs as follows:

Pandalus borealis Kröyer
P. hypsinotus Brandt
P. meridionalis Balss
P. kessleri Czerniavski
P. nipponensis Yokoya

These species are all important edible prawns, and all are found in northern Japan with the exception of one species population of *Pandalus nipponensis*. With regard to larval culture, three of these species have been studied by Japanese researchers in northern districts (Kurata, 1955 and 1964; Kashiwagi and Ohkawa, 1973; Motowo, et al., 1975; Ohmi and Yamashita, 1978).

Pandalus borealis Kröyer.

This prawn is distributed on the northern Pacific and Atlantic coasts and around the Arctic Ocean. In Japan it is found in deep coastal waters off Hokkaido and in the Sea of Japan. Total catches of the prawn from all these Japanese fishing grounds have recently amounted to about 2,000 tons annually.

The hatched larvae of the species were fed on three different diets: a) the rotifer, *Brachionus plicatilis*, b) the brine shrimp, *Artemia salina*, and c) larvae of the sea urchin, *Hemicentrotus pulcherrimus*, at a seawater temperature of 3°C on the coast of Noto Peninsula, located in the middle of the Sea of Japan coast by Motowo, et al. (1975). The survivorship of the larvae in 42 days of culture was highest (25 percent) for those fed on the rotifer.

Ohmi and Yamashita (1978) studied the optimal temperature for rearing larvae of this prawn on the coast of Volcano Bay, Hokkaido and reported that the water temperature of 9°C was optimum, giving an 8 to 13 percent survivorship rate for 60 days of rearing.

Pandalus hypsinotus Brandt.

This prawn inhabits deep-sea areas of the Bering Sea as far as Alaska, and from the southern coast of Hokkaido to the middle region of the Sea of Japan. It is edible on the coast of the Hokuriku region.

Ohmi and Yamashita (1978) contributed to the knowledge on rearing this prawn. The op-

timal temperature for rearing the larvae was 8 to 9°C. Hatched larvae were fed on three kinds of diets, brine shrimp, artificially synthesized zooplankton, and formula food for fish fry. Those fed on the brine shrimp diet showed the highest survivorship (18 to 20 percent) and finished the sixth molting in 60 days of culture. Larvae fed on formula food (TP-2) and on the artificial zooplankton showed only 6 percent survivorship and only a few individuals finished the sixth molting. The author pointed out that aeration was useful for raising the survivorship during rearing.

Pandalus kessleri Czerniavski

This prawn inhabits rather shallow water off the Kurile Islands, Hokkaido and northern districts of Honshu, on both the Pacific and the Sea of Japan coasts.

Kashiwagi and Ohkawa (1973) cultured adult females having fertilized and developing ova in a double-net crawl in shallow coastal waters in Yamada Bay, Sanriku District, employing minced fish-flesh as food. The fine outer net of the crawl (25 x 23 meshes per inch) contained the hatched larvae, while the ovigerous adult females were retained in the inner net (5 x 5 meshes per inch). The bottom of the net was covered with artificial marine algae made of vinyl chloride. The relationships between the number of artificial marine algae and the survivorship rate of hatched larvae were clear. The results obtained in the experiments indicated that cannibalism was one of the most important factors controlling the survivorship of hatched larvae.

Materials and Methods

The ovigerous female prawns used for the present experiments were obtained using a basket net on the continental slope of Suruga Bay at a depth of 460 meters on 5 February 1980. These ovigerous females were cultured in aquaria in the Marine Science Museum of Tokai University. From 6 March to the end of March, larvae hatched out intermittently. The environmental conditions during the experiment were 10.6°C water temperature, pH 7.7, 94 to 95 percent saturation dissolved oxygen, and 33.84 to 34.11 percent salinity. Also NO_2-N (nitrite—nitrogen) was 0.003 to 0.015 ppm, and NH_4-N (ammonium—nitrogen) was 0.002 to 0.003 ppm.

The water temperature for rearing larvae was determined to be 10°C based on the results of preliminary experiments carried out in the previous year. The aquarium for rearing was made of glass with a capacity of 10 liters. A filter tank of the same capacity was set up and connected to the rearing tank by a siphon. The water in the tank was allowed to circulate. Larvae with densities of 60 (A series) and 30 (B series) were contained in each aquarium.

Minced flesh of the little clam, *Tapes philippinarum*, was supplied daily at about the same weight as the body weight of the larvae in their molting stage. The wet weight of the body was measured when they completed molting, and at the same time the survivorship rate was recorded.

Results and Discussion

Growth of Larvae Following Molting

The process of growth of the larvae following molting is shown in Figure 1. In the figure, the abscissa shows the stage of the larvae, the left ordinate shows carapace length and the right ordinate shows wet body weight. The body length, that is the length from the base of the ocular peduncle to the end of the telson, in their first, third and sixth stage, was about 5.7, 6.9 and 11.1 mm, respectively.

In the first and second stage, the segmentation of pereopods was imperfect and the telson and pleopods were not separated. In the third stage, formation of the telson and the tail fan

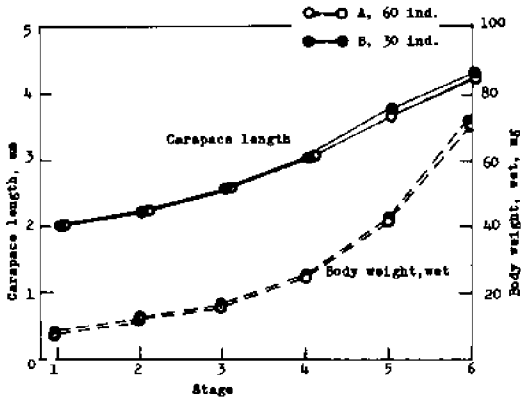

Figure 1. Relationship between the molting stage of larvae and the carapace length (left ordinate, solid lines), and relationship between the molting stage and the wet body weight of larvae (right ordinate, broken lines). Open and solid circles show the A series (60 larvae reared in 10 l of seawater) and the B series (30 larvae in 10 l), respectively.

was nearly completed. The pleopods were almost completed in the fourth stage (Figure 2). In the process of the experiments larvae finished the sixth molting and reached the seventh stage by the end of the 55 days of rearing. At this point in time, the larvae had about the same morphological characteristics as those of adult individuals.

The velocity of development was about equal with the three Japanese species belonging to the genus *Pandalus* mentioned above. Interrelations between the molting stage and the formation of appendages of larvae obtained in the experiments were about the same as the results obtained by the studies on *Pandalus borealis* by Berkeley (1930) at British Columbia, Canada, and on *P. kessleri* by Kurata (1955 and 1964) at Hokkaido, Japan. Kurata (1964), however, pointed out that the relationship between the formation of appendages and the

Figure 2. Larvae from the top picture to the bottom showing the first to the sixth molting stage. The telson and the tail fan are nearly completed at the third molting stage and the abdominal appendages (pleopods) are nearly formed at the fourth molting stage. The scale showing in the right bottom of the picture "5" indicates 5 mm in length.

molting stage is not always fixed, but the formation of appendages is changeable depending upon the environmental conditions for molting.

The change of water quality from 11 March at the start of the experiment, to 4 May at the completion of the experiment, is shown in Figure 3.

Survivorship Rate of Larvae

The survivorship rate of larvae at the completion of molting is shown in Figure 4. The ordinate shows the survivorship rate in percentage against the total number of larvae (60 in A series, 30 in B series) at the start of rearing. Similar to Figure 1, the white circle shows the ratio for A series and the solid circle for B series. Figure 4 indicates that the mortality of the larvae in the first molting stage is as high as 12 to 25 percent, whereas the mortality from the second molting stage to the end of the fifth molting stage is only 22 to 25 percent. From this fact, it is known that the mortality is high in their early stage of development. No significant difference was confirmed in the mortality between series A and B.

Estimation of NH_4-N and NO_2-N was most useful for the monitoring of water quality for raising survivorship rates of rearing larvae in the experiment; i.e., optimum ranges for larvae were under 0.4 ppm in NH_4-N and 0.1 ppm in NO_2-N concentrations.

Ingestion Rate of Larvae When Brine Shrimp Nauplii Were Fed

Three beakers were filled with seawater and aerated. Then four larvae, 24 hours after

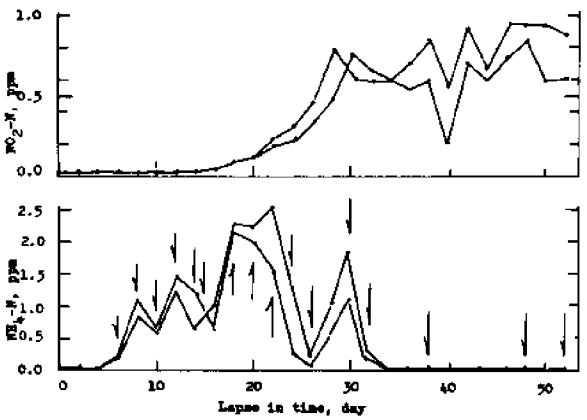

Figure 3. Prevalence of NH_4-N and NO_2-N in the seawater of the A series of rearing larvae. The arrows indicate the time point of exchange of water. The same prevalence was also obtained in the B series.

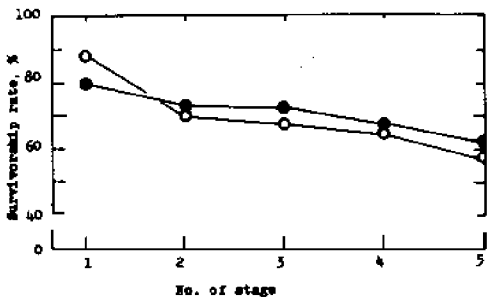

Figure 4. Percentage survivorship rate at the time when each molting stage was finished. Open and solid circles show the A series and the B series of rearing larvae, respectively.

hatching, were put in each of the beakers. In each of the beakers, some 200 brine shrimp nauplii which had been hatched from winter eggs and were frozen at -20°C were put in as food. After 20 hours, the number of brine shrimp nauplii eaten was counted. The experiments were repeated three times and the average numbers were plotted as shown in Figure 5 where the survivorship rate is shown on the left ordinate by a black dot and the number of nauplius ingested is shown on the right ordinate by a open circle. The lapse of time is shown on the abscissa of the figure.

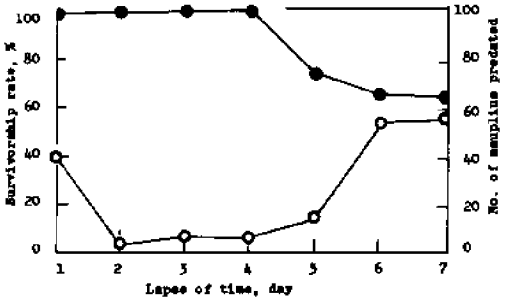

Figure 5. Percentage survivorship rate at each stage from hatching until all larvae finished the first molting (solid circles, left ordinate), and the relationship between the number of the *Artemia* nauplii taken by a prawn larva and the lapse of time in days of rearing (open circles, right ordinate).

The larvae began the first molting on the third day. The cumulative molting rate was 17 percent, 25 percent, 78 percent and 100 percent on the third, fourth, fifth and sixth days, respectively. That is, all the larvae reached the second stage by the sixth day. The conversion efficiency of food, the ratio between the increase of the body weight of prawn larvae and the weight of nauplius ingested, was about 22 percent.

It seems that larvae of *Pandalus nipponensis* enter bottom life right after the hatch and they do not swim in the middle layer of water. Relating to this behavior, it was found that they never ate the live brine shrimp nauplii supplied in the aquaria. Therefore, in the present experiment instantly frozen brine shrimp nauplii were given to the initial stage of larvae, and then minced flesh of the little clam was fed for later culture. This habit was not observed in other species of the genus *Pandalus*.

Egg Size of an Ovigerous Female

The size of an egg rapidly increased in the ten days before hatching. The increase was especially remarkable in the longer diameter. The longer diameter of the egg increased from 2.4 mm to between 2.6 and 2.7 mm in the first month after the ovigerous female was captured. The shorter diameter of the egg increased only from 1.8 to 1.9 mm to 1.9 to 2.0 mm. However, during the ten days before hatching, the longer diameter of the egg reached as long as 3.1 to 3.2 mm, whereas the shorter diameter became 2.0 to 2.1 mm. Hence, the longer diameter increased very rapidly, and the egg became oval.

Number of Eggs of an Ovigerous Female

The carapace length and wet weight of 22 ovigerous females were 30.25 to 37.20 mm and 25.0 to 40.0 grams, respectively. The number of eggs was between 455 and 762. Generally speaking, present species in which diameter of eggs is large, carry small numbers of eggs, while the species having eggs of shorter diameter carry larger numbers of eggs. Allen (1963) reported that in the case of *Pandalus borealis*, the female with a body length of 125 mm had as many as 1,300 eggs with their diameter around 1.1 mm. Also, Kubo (1951) reported a case where *P. kessleri* had 400 to 700 eggs with a longer diameter of 2.2 mm.

In conclusion, the present species is considered an allied species of *P. kessleri* distributed in Hokkaido.

Acknowledgement

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Section IX
Diseases and Their Distribution in Pacific Salmon

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Environmental Gill Disease (EGD): What It Is and What to Do About It

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Description and Significance

Environmental gill disease (EGD) is a subacute to chronic noninfectious disease of juvenile fish being raised under intensive culture conditions. It has a complex etiology with the direct cause considered to be accumulations of sufficient levels of free (undissociated) ammonia and/or suspended organic or inorganic solids to cause chemical and physical irritation of the gill tissues. Indirect causes of disease are feeding rate (lbs of feed/100 lbs of fish), fish density (lbs of fish/ft^3), dissolved oxygen levels, alkalinity, temperature, and pH of the water.

Histopathologically, the lesions are restricted to the lamellar epithelia unless the condition is further complicated by an infectious process. According to interpretation of available information, the pathologic process is initiated with hypertrophy of the lamellar epithelial cells. The sequence of subsequent changes is obscure but the following changes have been recorded: separation of the lamellar epithelium from the capillary endothelium with accumulations of fluid; local hyperplasia of the lamellar epithelium; general hyperplasia of the lamellar epithelium with partial to complete occlusion of the interlamellar spaces; bulbous engorgement of the lamellae with subsequent rupture and frank hemorrhage.

Clinically, the fish exhibit increased respiratory activity, anorexia and lethargy. The mortality rate in uncomplicated EGD is seldom very high. Its main effects are reduced growth rates, reduced dietary efficiencies, and increased production costs. However, uncomplicated EGD is more the exception than the rule. The condition (often undetected) frequently is the harbinger of infectious processes caused by cutaneous and systemic bacteria and by external protozoans and metazoans. All of which are frequently typified by extremely high morbidity and mortality rates.

The foregoing description of EGD is based upon a collation of the observations and interpretations of Klontz, et al. (1980), Wood and Yasutake (1957), Smith and Piper (1975), Burrows (1964), Smart (1976), Bullock (1972), Wales and Evins (1937), Eller (1975), Hartman (1979, unpublished), Westers and Pratt (1977), Larmoyeux and Piper (1973), Wood (1968 and 1974) and Snieszko (1974).

Unfortunately, there are no pathogenetic descriptions of EGD nor are there studies reported in which fish-generated ammonia-N and fecal solids have been quantitatively assessed insofar as their individual or collective roles in the process. The majority of the studies have dealt with the toxicological effects of introduced ammonia-N on the gill tissues. Brett and Zala (1975) have quantitated the ammonia excretion by sockeye fingerlings but did not correlate this with any pathological changes in gill tissues. From their data, we concluded that sockeye salmon fingerlings, at any rate, and perhaps salmonids in general can

generate sufficient excretory products to create clinical EGD. This conclusion is supported by the observations reported by Smith and Piper (1975) and by McLean and Fraser (1974).

The major pathophysiological alteration resulting from the thickened gill lamellar epithelium is reduced oxygen uptake which decreases the ability of the affected fish to withstand the rigors of a stressful situation, no matter what the cause. Wedemeyer (1970) and Snieszko (1974) have independently and comprehensively described the potential of stress-activated clinical disease caused by infectious agents.

In a survey of public and private salmonid hatcheries in Idaho, Klontz (1973) documented that more than 50 percent of the facilities visited reported having had significant stress-related occurrences of infectious diseases. It was estimated that 40 to 50 percent of the annual mortality (often as high as 50 percent from eyed egg to release or processing) in fish hatcheries was directly attributable to the existence of EGD prior to the onset of the infectious process.

Treatment Methods

At this time in our research, we think the best way to control an occurrence of uncomplicated EGD is to withhold feed from the fish for at least 48 to 72 hours, that is if the fish are large enough to permit this practice. Fish smaller than 200 to 300 per pound should not have feed withheld for more than 24 hours. This method markedly decreases both solids and ammonia-N generation—the two major causes of EGD. It also reduces the oxygen demand by the fish.

The next step is to either increase the water flow to the pond or reduce the biomass in the tank or both. In our experience the major contributing causes to EGD are overloading ponds and insufficient water flows.

If the outbreak of EGD is complicated with one or more infectious agents—protozoa or bacteria—then specific therapy should be instituted. With respect to chemotherapeutic measures for EGD, we in the food fish-raising business are caught between the rock and the hard spot. To our knowledge, only salt has been approved by the FDA for external use in food fish. As anyone who has treated gill diseases in fish will relate, several chemotherapeutants not on the list of approved chemicals are used routinely in treating gill diseases of food fish. So, we are not going to recommend any specific "wonder drugs" to add to the water to treat gill disease. But, we will recommend a way by which the chances for therapeutic efficacy will be enhanced.

The first task is to make an assessment as to the nature of the gill disease, i.e., is it simple EGD or is it complicated with an infectious agent? If it is uncomplicated EGD, then withholding feed, reducing the biomass, and increasing the water flow will often suffice. There are no chemicals, to our knowledge, which will accomplish this task.

If there are infectious agents present, then a chemical treatment is warranted; however, the treatment for EGD must still be instituted. After choosing a particular chemical and selecting a dosage and time of exposure, it is strongly recommended that a bioassay be run to verify that the particular chemical and exposure time are effective in resolving the problem. Thus, fish must be examined before the bioassay and afterwards—preferably at the termination of the exposure period and two to four hours later—to determine the chemical effect. The bioassay method should indicate positive results before the method is applied to the entire lot. In our collective opinion, groups of fish are continually being treated and retreated without any regard for the efficacy. A decreasing mortality rate is not sufficient evidence by itself to demonstrate efficacy.

Prevention Methods

The most effective method to prevent EGD that we know of is to keep pond loadings so that

critical levels of oxygen tension are not present. In addition, feeding rates and feeding frequencies must be kept at levels where ammonia-N and fecal solids, plus uneaten feed, do not accumulate at tissue damaging and growth restrictive levels. Finally, water replacement times in raceways should be between 20 and 30 minutes. This provides for adequate water velocity to help reduce the ammonia-N and solids levels plus providing oxygen make-up.

With respect to pond loadings, the simplest and perhaps safest method to determine pond carrying capacity is that derived by Piper (1972). By using a temperature-elevation compensated table, the factor obtained is multiplied by the average fish length in inches and the water inflow in gpm, and the allowable (maximum) weight of that sized fish in the pond is derived. Other methods have been presented by Klontz, et al. (1978).

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Effects of Injection of Hormones on the Expression of Infectious Hematopoietic Necrosis Virus in Spawning Sockeye Salmon (*Oncorhynchus nerka*)

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Infectious hematopoietic necrosis (IHN) is a virus disease occurring in virtually all populations of sockeye salmon (*Oncorhynchus nerka*) (Amend and Wood, 1972; Guenther, et al., 1959; Grischkowsky and Amend, 1976; Parisot, et al., 1965; Watson, et al., 1954; R. S. Grischkowsky, unpub. data). While the disease has little apparent effect on naturally spawning populations, when infected feral fish are used as an egg source for a hatchery, high mortality rates in the hatched fry often result. This mortality rate is typically 90 percent positive during the period of an epizootic. Large scale losses on natural populations have been known to occur (Williams and Amend, 1976). Recently, approximately 14 million sockeye alevins have died from the disease during one year at three Alaskan hatcheries. The appearance of IHN virus in returning adult sockeye salmon is closely correlated with the spawning act (Mulcahy and R. S. Grischkowsky, unpub. data). Using ovarian fluid (OF) as a sample (Watson, et al., 1954) from carrier fish, the virus may be absent or present in lower titer before spawning occurs. We tested the use of sex hormones to slightly advance the spawning time of naturally infected sockeye salmon to determine if eggs could be obtained prior to the appearance of the virus in OF and as an attempt to provide rapid virus incidence determination prior to a hatchery egg take. Human chorionic gonadotropin (HCG—Sigma Chemical Co.) and follicle stimulating hormone (FSH—Sigma Chemical Co.) were used rather than using salmon pituitaries or commercially available carp pituitaries because we wanted to eliminate any possibility of introducing adventitious fish viruses into the feral salmon at the experiment site. Hormones have been used to artificially advance spawning in other fish species (Donaldson, 1973; Fontaine, 1976; Yamazaki, 1976).

Experiments were done at Lake Nerka in western Alaska. Slightly unripe female sockeye salmon were seined from Little Togiak River for the FSH experiment and from the north end of Little Togiak Lake for the HCG experiment. The fish were placed in pens on a gravel beach and each population separated into experimental and control groups of about 60 fish each. The adipose fins of the control fish were clipped as a marker. Fish weight averaged 2,100 g. Each experimental fish received either 1 I.U. of HCG or 10 I.U. of porcine FSH in 2.5 ml normal saline. Control fish received 2.5 ml of normal saline. Injections of hormones or saline were made into the dorsal musculature immediately anterior to the dorsal fin.

We attempted to obtain OF samples from each fish the day after injection and then every

other day for five (FSH experiment) or six (HCG experiment) days post-injection. After six days the fish and pens were destroyed by bears. Fish were not sampled if they had not sufficiently ripened to yield eggs or OF when pressure was applied to the abdomen. From each ripe fish, a few ounces of eggs and OF were stripped into a paper cup. The OF was decanted into a sterile tube which was then placed on ice.

Preliminary sample processing was done in the field and consisted of low speed centrifugation to remove debris and blood cells, and the addition of antibiotics (penicillin, streptomycin, gentamicin and mycostatin). Processed samples were shipped on ice by air to the laboratory and cell cultures were inoculated with all samples within seven days after they had been taken. Samples were tested for IHN virus by standard virology and cell culture methods (American Fisheries Society, 1974) using the fathead minnow (*Pimephales promelas*) cell line (FHM). The virus in positive samples was titered on FHM cells by the 50 percent tissue culture infectious dose (TCID) end point method of Reed and Muench (1938).

No significant advances in ripening of the injected fish were noticeable because they were very close to ripe when injected. For the HCG experiment, 88.7 percent of the samples from the injected fish and 91.8 percent of the samples from the control fish were IHN virus positive. The total percent positive samples from the FSH experiment was 82.8 percent for the hormone-injected group and 80.4 percent for the control group. The differences in the results of the two experiments were due to the use of different populations as sources of fish. These data indicated that the hormones, at the levels injected, did not appreciably induce or totally suppress the virus in the experimental fish.

The overall mean titer of all positive OF samples was $10^{6.11}$ $TCID_{50}$ per ml from the HCG-injected fish and $10^{4.94}$ $TCID_{50}$ per ml from the control group. This apparent difference is due to the presence in the HCG-injected group of one sample taken on the first day post-injection with an extremely high titer of $10^{7.75}$ $TCID_{50}$ per ml. This is ten times greater than the next highest titer of a sample from any group. In the FSH experiment, the means of all titers in each group were $10^{2.80}$ $TCID_{50}$ per ml and $10^{4.89}$ $TCID_{50}$ per ml for the FSH-injected and control groups, respectively.

Figure 1 shows the daily means of the OF virus titers. In the HCG experiment, the daily mean titers of the hormone injected group showed an apparent decrease over the first four days after injection. The control group titers remained constant. However, analysis of variance revealed no significant ($p = 0.10$) difference between the control and hormone-injected groups. A different pattern of virus expression is seen in the FSH experiment in both the hormone treated and control groups. The daily mean virus titers of the control group show a steady increase peaking on the fourth day. The hormone-injected group showed a fairly level daily mean titer over the course of the experiment. The maximum difference in titers between the FSH-injected and control groups occurred on the fourth day post-injection. This thousand-fold difference in mean titers was significant at the $p = 0.10$ level but not at $p = 0.05$ by analysis of variance. Injection of FSH apparently prevented the rise in titers which occurred in the control fish. The difference in expression of IHN virus seen between the control groups of the two different populations of salmon may be due to intrinsic differences in their expression of this disease, or the fish were at different stages in their spawning cycle (Mulcahy, unpub. data).

Although FSH-injection reduced the mean titers attained, it did not prevent the appearance of IHN virus, as judged by the identical percentage of positive samples from the control and hormone-treated groups. We examined how the hormones affected the distribution of virus titers by making frequency histograms (Figure 2) of the virus titers of both hormone-injected groups and their controls. HCG-injection did not alter the distribution of virus titers compared to saline-injected controls. However, injection of FSH appeared to prevent development of the highest virus titers. A total of 17.6 percent of the titers from the control group exceeded $10^{3.0}$ $TCID_{50}$ per ml, but only 4.1 percent of the FSH injected group ex-

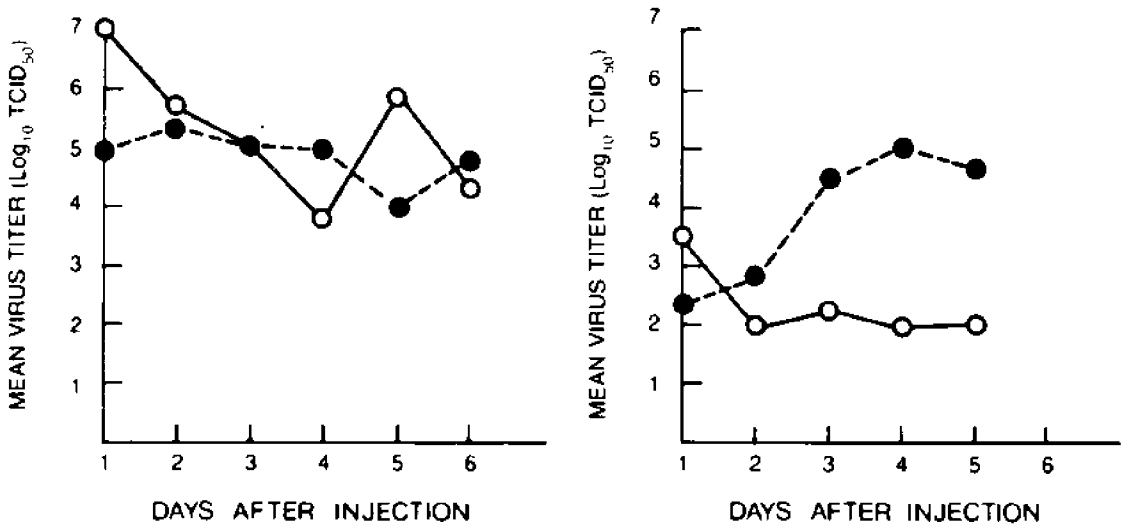

Figure 1. Daily mean IHN virus titers of ovarian fluid samples from fish injected with HCG (left) or FSH (right) and from respective saline-injected control fish. Symbols: (0————0) hormone-injected, (0- - - - -0) saline-injected.

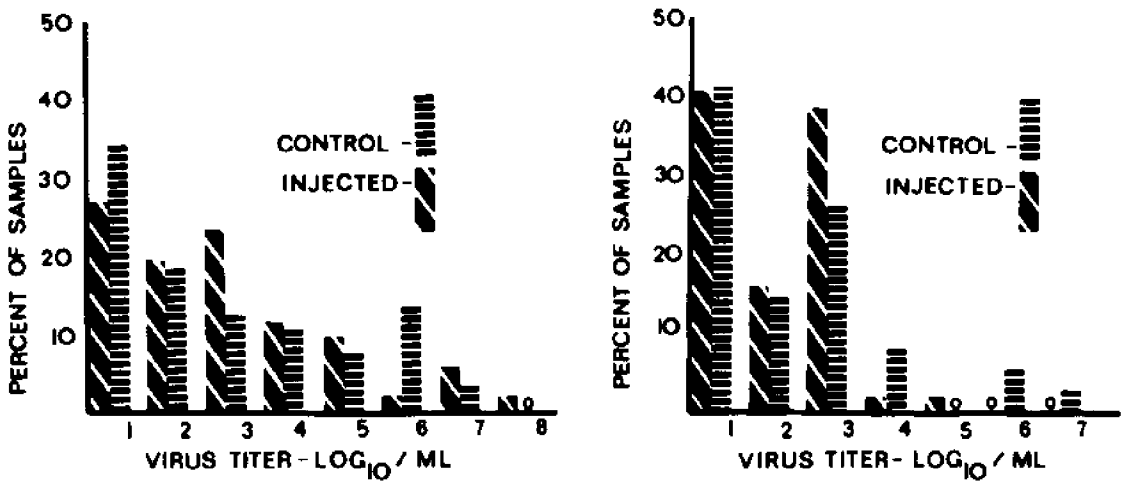

Figure 2. Frequency histograms of IHN virus titers in ovarian fluids of control (saline-injected) and HCG-(left) and FSH-(right) injected fish.

ceeded that level. Presently, we do not know the influence of virus titer on the efficiency of egg-associated transmission of IHN virus.

In *in vitro* tests using a fish cell culture system, FSH did not significantly alter the adsorption of (*epithelioma papillosum carpio* — EPC) IHN virus to the cells. At the levels tested (10^{-1}, 10^{-3}, 10^{-5}, 10^{-7} and 10^{-9} units per ml), there was no significant change in virus yield after six days exposure and no definitive results occurred.

Presently, the only effective means of avoiding the high mortality rate characteristic of IHN in hatchery reared fish is to avoid contact of the pathogen with the host. Clearly, this is difficult when all potential sources of sockeye salmon-eggs are naturally infected with virus.

Since these experiments represent only the second *in vivo* modification of the host-pathogen relationship with this disease (Amend, 1976), we feel this approach merits further effort.

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Viral Diseases of Salmonid Fish in Oregon

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Abstract

Selected populations of salmonid fish in the hatcheries, rivers and lakes of Oregon have been examined extensively for the presence of viruses pathogenic for these species. Two viruses have been isolated during the course of this investigation and both have caused significant mortality among alevin trout or salmon. Infectious hematopoietic necrosis (IHN) virus and infectious pancreatic necrosis (IPN) virus have also been recovered from adult salmonids which were carriers. Evidence for the presence of viral erythrocytic necrosis (VEN) virus has been found in red blood cells of fish taken in waters along the Oregon coast.

Introduction

With the recognition of virulent viruses in certain salmonid populations, it became important to obtain information concerning the prevalence and distribution of these agents in Oregon waters (Mulcahy, et al., 1980). Such information was needed in order to identify potential sources of virus-free eggs from wild fish, to avoid the transfer of fish carrying viruses to hatcheries or waters free from such agents, and to assess the possible effect of the viruses on the maintenance and welfare of Oregon salmonid fisheries. Accordingly, salmonid populations have been examined throughout the state over the 22 year period from 1958 through 1980, for the presence of viruses. Hatcheries, rivers and lakes have been included. It is the purpose of this report to provide information concerning the occurrence and distribution of salmonid viruses in the State of Oregon.

Infectious Hematopoietic Necrosis

The first occurrence of disease caused by what is now presumed to be IHN virus was observed in sockeye salmon (*Oncorhynchus nerka*) in hatcheries at Winthrop and Leavenworth, Washington, and was described by Rucker, et al. (1953). Watson, et al. (1954) reported further experimental evidence for the viral etiology of this disease. In 1958, an outbreak of disease with a high mortality rate appeared among young sockeye salmon at the Oakridge Salmon Hatchery in Oregon. Using primary fish cell cultures, an infectious agent was isolated from tissues of sockeye salmon (Pilcher and Fryer, 1980a). The disease was transmitted to juvenile sockeye salmon by injection of either cell culture fluids or bacterial-free filtrates from infected salmon tissues. J. W. Wood (personal communication) demonstrated the presence of a filterable agent in sockeye at the Oakridge Salmon Hatchery prior to these observations. Wood prepared homogenates of infected fish, passed this through a porcelain filter and injected the bacterial-free filtrate into healthy animals, producing death with signs typical of IHN. This work was accomplished about 1957. In 1967, Amend, et al. (1969) isolated a virus from disease outbreaks in rainbow trout (*Salmo gairdneri*) and sockeye

salmon in western Canada. Studies of these isolates indicated that they were similar to the virus recovered from sockeye salmon in Oregon. Pronounced cytotropism for the hematopoietic tissues of the host suggested the name "infectious hematopoietic necrosis" (IHN) for the disease.

The viral agent isolated from infected fish was found to replicate in sockeye salmon cell cultures, producing characteristic cytopathic effects (Wingfield, et al., 1969). The maximum rate of replication occurred in the temperature range of 13 to 18°C, and no replication occurred at 23°C, though host cells grew well at that temperature. Infectivity was destroyed by ether, indicating the presence of essential lipids. The virus replicated in the cytoplasm of fish cells in culture (McAllister, et al., 1974) and its genome was shown to be single-stranded ribonucleic acid (RNA). Examinations of IHN virus by means of electron microscopy revealed bullet-shaped virions with a mean length of 188 nm and a diameter of 70 nm (Amend and Chambers, 1970). These observations have been confirmed and have resulted in classifying it as a member of the rhabdovirus group, which contains bullet-shaped viruses. The morphology of the virions is shown in Figure 1.

Figure 1. Electron micrograph of infectious hematopoietic necrosis virus (IHNV). Bar is 200 nm.

In juvenile sockeye salmon, one of the most characteristic signs of infection was the presence of long, opaque, fecal casts trailing from the vent. Ascites, exophthalmos and hemorrhagic areas in the musculature adjacent to the dorsal kidney and spleen were also observed. Internal gross signs include a pale liver, kidney and spleen. Microscopic examination of the viscera shows extensive degeneration and necrosis in the kidney, spleen and pancreas; and the hematopoietic tissues of the kidney and spleen are most severely damaged (Amend, et al., 1969).

The appearance of IHN among hatchery stocks is often characterized by the explosive nature of the disease and the high mortality rate produced. Natural epizootics have occurred in juvenile rainbow and steelhead trout (*Salmo gairdneri*) as well as chinook (*Oncorhynchus tshawytscha*) and sockeye salmon, and cutthroat trout (*Salmo clarki*) have been experimentally infected. Coho salmon (*Oncorhynchus kisutch*) are resistant. The signs of the disease in chinook salmon are similar to those in infected sockeye salmon and rainbow trout, and the hematopoietic tissue of the kidney undergoes extensive damage (Yasutake, et al., 1965).

Infectious Pancreatic Necrosis

The disease, infectious pancreatic necrosis (IPN), was first described by McGonigle (1941) as an "acute catarrhal enteritis" in very young brook trout, *Salvelinus fontinalis*. The infectious nature of the disease was established by Snieszko, et al. (1959) and the virus was first isolated by Wolf, et al. (1960). A cytopathic effect (CPE) was produced by the virus in brook

trout cell cultures carried through nine serial passages. Culture fluids from the sixth and eighth passage produced the typical disease when fed to susceptible young brook trout.

Since this virus was first isolated by Wolf in 1960, numerous isolations have been made from infected salmonids by other investigators in North America, Europe and Japan (Pilcher and Fryer, 1980a). The virions of IPN are icosahedral with a mean diameter of 55 nm (Lightner and Post, 1969) and a capsomer count of 92. The viral genome is composed of two segments of double-stranded RNA (Dobos, 1976). IPN virus has the same size and shape, as those of the reovirus group, but lacks the inner capsid and its double-stranded RNA has only two segments instead of ten. The morphology of the virions is shown in Figure 2.

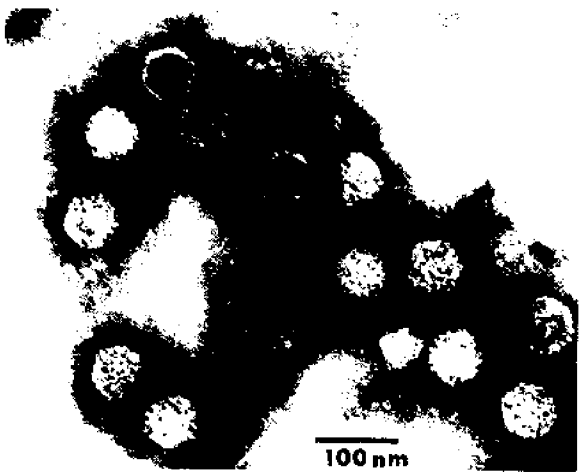

Figure 2. Electron micrograph of infectious necrosis virus (IPNV). Bar is 100 nm.

The first recognized epizootic of infectious pancreatic necrosis was reported in fingerling brook trout by Snieszko, et al. (1959). Since that time outbreaks have been described in rainbow, cutthroat, and brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*). A sudden increase in mortality is often the earliest sign of an IPN epizootic (Yasutake, 1970). Many of the infected fish exhibit an abnormal type of swimming, characterized by a corkscrewing or whirling motion. They may also show one or more of the following signs: dark coloration, exophthalmia or ascites. Internally, a mucoid plug in the stomach and anterior intestine is often present. A pale liver and spleen, petechiae in the caecal area, and an empty digestive tract are also characteristic.

Histologically, there is massive necrosis of the acinar cells of the pancreas, with pyknosis of nuclei and inclusions in the cytoplasm. Islet tissue may also show such changes. Pathological changes in the hematopoietic tissue of the kidney have been found frequently in terminal cases, resembling those found in early cases of infectious hematopoietic necrosis (Yasutake, et al., 1965).

Infectious pancreatic necrosis virus was detected for the first time in Oregon salmonids during 1971. Wild cutthroat trout in a small stream were serving as carriers of IPN virus. During the same year, IPN virus was isolated from adult coho salmon being spawned at the Bonneville Hatchery on the Columbia River (McMichael, 1974). These salmon also appeared to be healthy carriers of the virus.

Viral Erythrocyric Necrosis

Laird and Bullock (1969) described a pathological condition in the erythrocytes of three fish species, cod, *Gadus morhua*, seasnail, *Liparis atlanticus*, and shorthorn sculpin, *Myxocephalus scorpius*, collected from coastal waters of eastern Canada and northwestern United States. They observed by light microscopy, eosinophilic inclusion bodies measuring

up to 1 μm in diameter in the cytoplasm of infected erythrocytes. The nuclei of such cells became distorted, and a round clear vesicle developed within them, containing dense staining particles from 0.25 to 0.5 μm in diameter. They suggested a possible viral etiology, and named the disease piscine erythrocytic necrosis. Recently the disease has been referred to as viral erythrocytic necrosis (VEN).

Inclusions have also been observed with erythrocytes of the Atlantic herring, *Clupea harengus harengus* (Sherburne, 1973), and the blenny, *Blennius pholis* (Johnson and Davies, 1973). Sherburne (1977) disclosed the presence of the same type of inclusions in erythrocytes of the anadromous alewife, *Alosa pseudoharengus*, from Maine coastal streams. The same or very similar condition has been reported in Atlantic herring by Philippon, et al. (1977) and Reno, et al. (1978).

Walker (1971) found what appeared to be the same condition in two of 18 cod from waters off the coast of New Brunswick. Electron microscopic examinations of erythrocytes revealed the presence of large cytoplasmic particles with hexagonal profiles and center spacings of 350 nm similar to virions of the iridovirus group.

Walker and Sherburne (1977) indicated that a cytoplasmic structure adjacent to a group of the virions, which they called the viroplasm, was Feulgen positive, and concluded from this that the hexagonal particles represented a DNA virus. This evidence however must be considered presumptive.

Evelyn and Traxler (1978) found VEN in chum and pink salmon, *Oncorhynchus keta* and *Oncorhynchus gorbuscha*, taken off the Pacific coast of North America in 1976. It was encountered frequently in these species held in net pens in seawater, and seemed to be most severe during the summer. Mortalities caused by VEN were low, i.e. up to 0.3 percent.

These workers also demonstrated the infectious nature of VEN by injecting uninfected chum and pink salmon with homogenized kidney and spleen from infected chum salmon. Nine of ten pink salmon inoculated with fourth passage material showed infection within three weeks, as determined by examination of blood. The same inoculum failed to infect chinook, coho or sockeye salmon. Attempts to isolate the virus by inoculating rainbow trout gonad, chinook salmon embryo, and fathead minnow cell cultures, were unsuccessful. The average measurement between opposing apices of the hexagonal and pentagonal particles in the blood of experimentally infected fish was 190 nm. The morphology of the virions and the inclusion bodies within erythrocytes are shown in Figures 3A and 3B.

The chief pathological change observed in VEN infected fish has been abnormal erythrocytes, which contain eosinophilic inclusion bodies and distorted nuclei. The latter may include a round clear vesicle enclosing a number of dense staining particles from 0.25 to 0.5 μm in diameter. Evelyn and Traxler (1978) found that infected chum and pink salmon were anemic, and histologically the kidney showed extremely active hematopoiesis.

Materials and Methods

Infectious Hematopoietic Necrosis and Infectious Pancreatic Necrosis Viruses

The number of fish sampled from a population was generally in accordance with Ossiander and Wedemeyer (1973) for detection of a minimum 5 percent carrier incidence. Fewer samples were taken if fish showed external signs of disease and a viral etiology was suspected. The tissues and fluids for examination were selected to maximize the detection of virus. The size and sexual maturity of the fish also helped to determine the type of sample for analysis (Fryer, et al., 1979). Whole fish, visceral organs or gonadal fluids were prepared for analysis as pools from five fish by standard methods (Fish Health Section, American Fisheries Society, 1979).

Gonadal fluids were diluted 1:5 while tissue homogenates were diluted 1:100 in buffered saline, and inoculated onto fish cell cultures. These cell cultures were grown in Eagle's

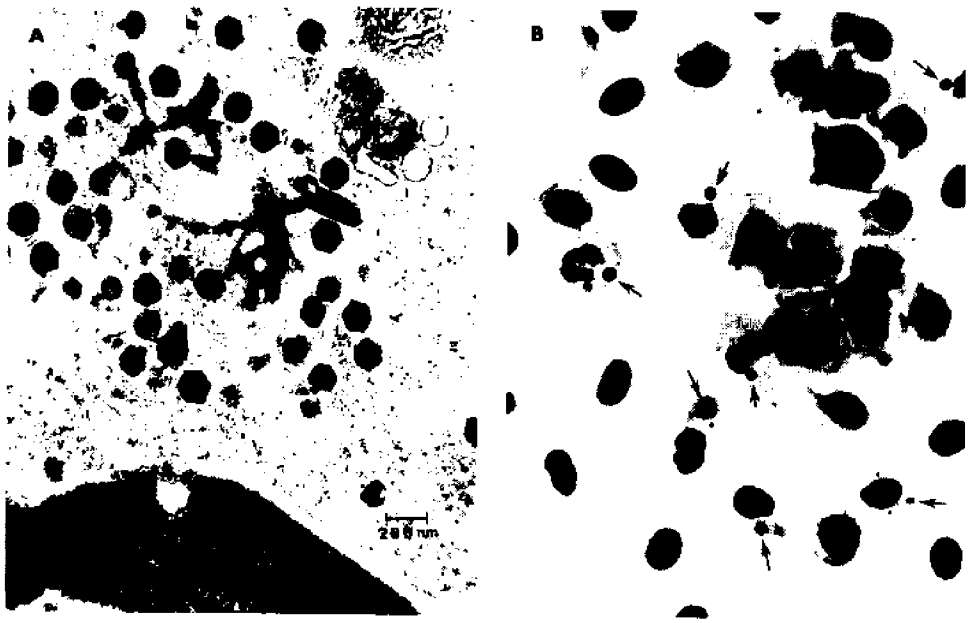

Figure 3. Viral erythrocytic necrosis (VEN): a) electron micrographs of virions within fish erythrocytes, b) light micrograph showing inclusions within infected erythrocytes.

Minimum Essential medium supplemented with 10 percent fetal calf serum (MEM-10) and buffered with sodium bicarbonate and incubated at 16 to 18°C. Aliquots of sample suspension were inoculated onto cell monolayers that were approximately 75 percent confluent. Ninety-six well flat bottom culture plates were most often used, however, 12 and 24 well plates and 25 cm^2 plastic flasks were also frequently employed.

The chinook salmon embryonic (CHSE-214) cell line (Fryer, et al., 1965) was used throughout this work. Duplicate samples were always inoculated onto a second cell line, usually the steelhead trout embryonic line STE-137 (Fryer, et al., 1965). The rainbow trout gonad (RTG-2) (Wolf and Quimby, 1962), fathead minnow (FHM) (Gravell and Malsberger, 1965) and epithelioma papillosum cyprini (EPC) (Tomasee and Fijan, 1971) cell lines have also been utilized.

Inoculated cell cultures were examined daily for 14 days by microscopy for possible development of CPE. Samples were considered negative after 14 days of incubation if no CPE was observed. During the past three years culture fluids from such preparations were further analyzed by inoculating them onto fresh cell monolayers and incubating them for an additional 14 days (blind pass).

If CPE was observed, the culture fluid was diluted 1:100 in buffered saline and inoculated onto fresh cells to a final dilution of 1:1,000. If CPE again developed, another 1:100 dilution of the culture fluid (dilution of original sample now 1:1,000,000) was again inoculated onto a fresh cell culture. Continued cell destruction at this dilution distinguishes a replicating agent (virus) from toxic substances.

Identification of all viral isolates was confirmed by serum neutralization using viral specific antiserum taken from rabbits hyperimmunized against either IPN or IHN virus (Mulcahy, et al., 1980). This procedure always included known virus as controls.

Viral Erythrocytic Necrosis

Blood samples were collected in heparin (150 units/ml) and smears were made on clean

glass microscope slides. The red cells were fixed for five minutes in absolute methanol. They were stained with Giemsa and examined by light microscopy for inclusions within erythrocytes (Figure 3B). The presence of inclusions was presumptive evidence of VEN infection. Additional evidence for VEN infection was obtained by electron microscopical examination of erythrocytes.

Results

Infectious Hematopoietic Necrosis

Infectious hematopoietic necrosis virus was the first fish virus to be isolated and identified from fish in the State of Oregon (Figure 4). The agent was recovered from juvenile sockeye salmon undergoing a severe epizootic at the Oakridge Salmon Hatchery in 1958 (Wingfield, et al., 1969; Table 1). It is thought that the source of the virus was unpasteurized sockeye salmon viscera from Puget Sound in the State of Washington which had been incorporated into the diet of the fish. The feeding of such preparations was discontinued many years ago and only pasteurized fish products are now included in the diets of salmonids in the Pacific Northwest.

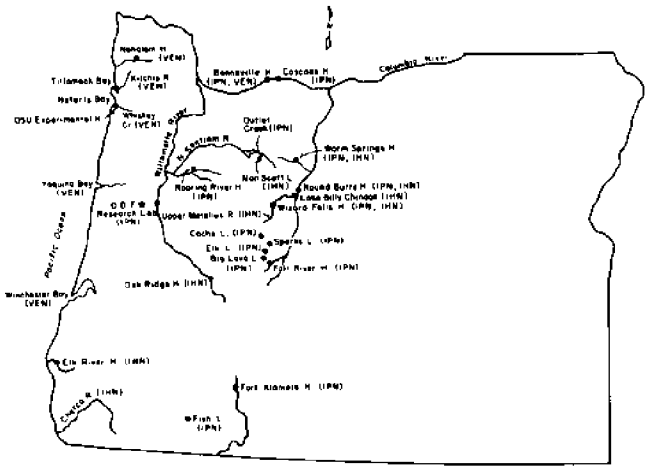

Figure 4. Locations of hatcheries, lakes and streams where IHN or IPN virus or both were isolated; and coastal waters where VEN virus was found.

The second known occurrence of IHN in Oregon was at a private lake on the Willamette River drainage in August of 1971 (Table 2). An epizootic in juvenile rainbow trout occurred at Nan-Scott Lake with signs of disease characteristic of IHN. These fish were hatched from eggs imported from a private trout farm in eastern Washington and it is believed that the fish were carrying the virus at the time they were released into the lake.

During March of 1975, rainbow trout and kokanee salmon (landlocked *Oncorhynchus nerka*) juveniles began dying in large numbers at Wizard Falls Hatchery on the Metolius River in central Oregon. The trout were derived from Roaring River Hatchery broodstock and the kokanee were spawned from feral fish at two central Oregon lakes.

Extensive examination of moribund fish revealed the presence of both IHN and IPN virus, in some cases in the same fish (Mulcahy and Fryer, 1976). Pathologically, IHN virus appeared to be the most important cause of mortality. The source of either virus in this case

Table 1. Oregon hatcheries where infectious hematopoietic necrosis or infectious pancreatic necrosis viruses have been isolated from salmonid fish.

Hatchery	Species and stage of development[1]	Date of first isolation	Virus	Fish mortality caused by viruses
Oakridge	SS Juv	9/58	IHN	Yes
Bonneville	Co Ad	10/71	IPN	No
	Co Al	2/72	IPN	No
Cascade	Co Juv[2]	2/72	IPN	No
	Co Ad	10/72	IPN	No
Round Butte	ChS Ad	8/73	INH	No
	ChS Ad	9/73	IPN	No
	StS Al	4/75	IHN	Yes
	StS Ad	1/76	IHN	No
Fall River	BT Juv	6/73	IPN	Yes
	Rb Juv	6/73	IPN	Yes
Roaring River	Rb Ad Br	12/73	IPN	No
Oregon Dept. of Fish & Wildlife Research Lab., Corvallis	ChS Juv	12/73	IPN	No
Fort Klamath	Br Juv	1/74	IPN	No
Wizard Falls	BT Juv	1/74	IPN	Yes
	K Juv	3/75	IHN	Yes
	Rb Juv	3/75	IPN/IHN	Yes
Elk River	ChF Ad	1/76	IHN	No
	ChF Juv	4/76	IHN	Yes
Warm Springs[3]	StS Ad	3/79	IHN	No
	StS Juv	9/79	IPN	No

[1]The following abbreviations are used in the Tables of this report.

Abbreviations for fish species.

Br brown trout
BT brook trout
Ct cutthroat trout
K kokanee salmon
Rb rainbow trout
ChF fall chinook salmon
ChS spring chinook salmon
Co coho salmon
StS summer steelhead trout
StW winter steelhead trout
ChuS chum salmon
PaH Pacific herring

Abbreviations for stage of development.

Al alevin
Juv juvenile
Ad adult
Ad Br adult brood trout

[2]Fish were from Bonneville Hatchery, Oregon.

[3]Virus isolations at this site were made by S. Leek, U.S. Fish and Wildlife Service.

Table 2. Oregon lakes and rivers where infectious hematopoietic necrosis or infectious pancreatic necrosis viruses have been isolated from salmonid fish.

Location	Species	Date of first isolation	Virus
Nan-Scott Lake	Rb Ad	8/71	IHN
Outlet Creek for Nan-Scott Lake	Ct Ad	9/71	IPN
Fall River	BT Ad	6/73	IPN
Fish Lake	BT Ad	9/73	IPN
Big Lava Lake	BT Ad	9/73	IPN
Cache Lake	BT Ad	7/74	IPN
Sparks Lake	BT Ad	7/74	IPN
Upper Metolius River	K Ad[1]	10/75	IHN
Elk River	ChF Ad	1/76	IHN
Chetco River	ChF Ad	12/77	IHN
Lake Billy Chinook	K Ad[1]	5/78	IHN

[1]Landlocked sockeye, *Oncorhynchus nerka*.

is difficult to assess. About one month after the Wizard Falls epizootic, juvenile steelhead trout that were hatched and being reared at Round Butte Hatchery underwent an epizootic attributed to IHN disease. The proximity of Wizard Falls and Round Butte hatcheries suggests a possible etiological relationship for these two occurrences of IHN.

Because of the 1975 epizootics at Wizard Falls and Round Butte hatcheries, the possible role of the kokanee salmon population of the Metolius River as a source of the virus was considered. Thirty adult kokanee were collected from the spawning grounds on the Upper Metolius River and IHN virus was isolated from these specimens. However, the role of these virus carrying fish in the 1975 epizootics is not clear.

The only other hatchery in which IHN virus was detected during the course of this work was the Elk River Hatchery near the Pacific coast in southern Oregon. In January 1976, 50 adult fall chinook salmon were examined, using both visceral and ovarian fluid samples. Infectious hematopoietic necrosis virus was isolated from all of the fluid and several of the visceral samples. Several weeks after the eggs taken from these fish hatched, the fry suffered an epizootic caused by the virus. The adult females were free of symptoms and were harboring the virus in a carrier state. It was again isolated from adult fall chinook at the hatchery in each successive year from 1976 through 1980, although never at the high incidence observed in the 1976 brood.

Infectious Pancreatic Necrosis

Infectious pancreatic necrosis was detected for the first time in Oregon salmonids in September of 1971. In August, the epizootic of IHN among rainbow trout in Nan-Scott Lake was in progress. It was of interest to determine whether native stocks of fish inhabiting the creek into which the lake drained might also be infected with IHN virus. A sample of 11 adult cutthroat trout was collected from these streams and tested for presence of the virus. No IHN virus could be detected, but instead these fish were found to be carriers of a strain of IPN virus (Table 2).

A second isolation of IPN virus was made in October of 1971 at the Bonneville Hatchery. Visceral samples from 60 Columbia River coho salmon were analyzed and IPN virus was detected in two of the five fish pools. These adult coho were apparently healthy carriers of this virus (Table 1). Adult coho and fall chinook salmon were examined at this hatchery again in September and October of 1972 though no further virus isolations were made. However, specific antibody against IPN virus was detected in both species, indicating that the fish at some time had contact with the virus.

In February 1972, two examinations were made of juveniles hatched from eggs taken at Bonneville from infected coho. On both occasions the fish were found to be infected with IPN virus, however, mortality attributable to it was not observed among the juveniles in the hatchery. On 21 March, three weeks after the second isolation of virus, a third examination was made. No virus was recovered but antibody against the coho strain of IPN was detected. Samples taken from adult coho spawned at Bonneville from 1972 to 1980 have not yielded virus.

In October 1972, IPN virus was isolated from adult coho salmon returning to Cascade, another of the Columbia River hatcheries (Table 1). Several of these fish apparently harbored the virus, and once again were serving as carriers. Although offspring from this spawning were isolated in the hatchery and repeatedly examined, no viral agents were recovered.

In May and June of 1973, the first hatchery epizootic of infectious pancreatic necrosis ever reported in Oregon took place. This occurred at the Fall River Hatchery, Oregon Department of Fish and Wildlife, and the population of fish chiefly affected were juvenile eastern brook trout (Table 1). Mortality was high and the symptoms were characteristic of IPN infection. No bacterial or protozoal pathogens were found and IPN virus was isolated and identified from moribund animals. Juvenile and yearling rainbow trout being reared at the hatchery

were without external signs but the virus was recovered from these fish. At the time of the epizootic, the virus was also isolated from brook trout taken from a stream at sites both below and above the hatchery, though it was not detected in either rainbow or brown trout taken from these sites at that time.

As a result of this epizootic, an examination of certain central Oregon salmonid populations for viral agents was undertaken in the summer of 1973. This survey detected the presence of IPN virus in eastern brook trout in Elk, Big Lava, and Fish lakes.

In September 1973, Fall River and its tributaries were treated with rotenone to eliminate the fish populations inhabiting these waters. Thorough disinfection of the hatchery facilities was carried out, and operation of the hatchery was discontinued for a period of nine months.

Three other isolations of IPN virus were made during 1973. In September, 30 adult spring chinook salmon were examined and tested at Round Butte Hatchery, and were found to be carriers of the virus (Table 1). In December, 60 adult rainbow trout at the Roaring River Hatchery were sampled and the ovarian fluids divided into 12 five-fish pools. One of the 12 yielded an isolate of IPN virus. No outbreak of disease is known to have occurred among fry from their eggs held at the hatchery. The virus was isolated from a sample of 60 juvenile spring chinook salmon being held at the Department of Fish and Wildlife Research Laboratory, Corvallis, Oregon. They did not show signs of disease, and were apparently carriers.

In January of 1974, the second IPN epizootic in an Oregon hatchery occurred. Eggs taken from East Lake brook trout in the fall of 1973 were transported to Wizard Falls Hatchery. In January the trout hatched from these eggs began to die in large numbers with symptoms suggesting a virus infection. Infectious pancreatic necrosis virus was isolated from several samples of moribund fish and its identity confirmed by reaction with specific antibody. Almost 700,000 juvenile brook trout were either killed by the disease or had to be destroyed. The ponds that had held the infected fish were disinfected and allowed to dry, and the hatchery building was partially disinfected. A group of these fish that had been removed from the hatchery as eggs and placed in the laboratory for experimental use were tested, but no virus was isolated.

While the Wizard Falls IPN epizootic was in progress, 50 juvenile brown trout from Fort Klamath Hatchery were collected and tested for the possible presence of a virus. These fish were not experiencing abnormal mortality despite the fact that they were found to be harboring IPN virus. The source of the eggs from which the brown trout hatched was also East Lake. The fact that both these fish and the brook trout in the IPN epizootic were derived from East Lake fish casts doubt on the suitability of this lake as an egg source. This was despite the fact that many adequate samples of East Lake brook and brown trout had been tested during 1973 and no fish virus had ever been detected.

During the summer of 1974, a survey was carried out for fish viruses in the lakes of the Cascade mountains in central Oregon. The presence of IPN virus in the brook trout of Elk and Big Lava lakes has already been mentioned. Only three other lakes of the considerable number examined yielded any virus isolates. These were Cache Lake, Sparks Lake and Fish Lake. In each case the virus isolated was IPN and the salmonid species was brook trout.

In March 1975, another epizootic occurred at Wizard Falls Hatchery. Both IPN and IHN viruses were isolated from the affected fish. Details of this epizootic were discussed in the preceding section concerning isolations of IHN virus.

All infected stocks of fish at Wizard Falls Hatchery in 1975 were destroyed and the hatchery was disinfected. No viral epizootic has occurred at this hatchery since. Furthermore, populations of kokanee salmon, brook and brown trout, and Atlantic salmon at the hatchery have been examined repeatedly for the possible presence of fish viruses, with negative results.

Viral Erythrocytic Necrosis

The presumed virus of viral erythrocytic necrosis has not been isolated in cell culture (Pilcher and Fryer, 1980b). The virus has only been detected by the typical pathological changes produced in erythrocytes, and the presence of the hexagonal or pentagonal particles seen by electron microscopy in the cytoplasm of infected cells (Figures 3A and 3B).

The virus was first identified in Oregon by Marie Philippon-Fried in diseased adult chum salmon returning to the OSU Experimental Hatchery at Netarts Bay in 1976 (Table 3). It was also observed in this species in 1978 and 1979. These observations were confirmed by Olson (1977). Philippon-Fried also found VEN in 1976 in chum salmon from the Kilchis River, which flows into Tillamook Bay, Oregon and in Pacific herring, *Clupea harengus pallasi* from Yaquina Bay. Recently, scientists (A. Amandi and J. S. Rohovec) from the fish disease laboratory (Department of Microbiology) at Oregon State University have observed heavily infected Pacific herring from Winchester Bay, Oregon (Figure 4).

The virus has also been recently (1979) detected in adult coho salmon returning to Nehalem Hatchery. In addition, chum salmon alevins obtained at the Hoodsport Hatchery, Washington, and reared at Bonneville Hatchery, Oregon were found to be infected (Table 3).

Table 3. Locations where viral erythrocytic necrosis (VEN) was detected by light and electron microscopy within erythrocytes of fish.

Location	Species	Date of first detection
OSU Experimental Hatchery at Netarts Bay	ChuS Ad	11/76
Kilchis River	ChuS Ad	12/76
Yaquina Bay	PaH Ad	3/77
Nehalem Hatchery	Co Ad	10/79
Bonneville Hatchery[1]	ChuS Al	2/80
Winchester Bay	PaH Ad	8/80

[1]Diagnosis based on light microscopy only.

Discussion

The importance of determining virus infected fish populations was to 1) identify virus-free egg sources and 2) prevent the spread of viruses from infected stocks to uninfected fish in other geographical areas.

Infectious hematopoietic necrosis virus has been repeatedly isolated from salmonid populations in only two locations in the State of Oregon. Kokanee salmon that spawn in the Upper Metolius River and the steelhead trout that return to Round Butte Hatchery are chronically infected with IHN virus. Because these two groups share the same upper Deschutes River watershed, there is a possibility that IHN virus released by them contributes not only to the transmission of infection within a single population but also between the two species.

In addition, IHN virus is enzootic among fall chinook in the Elk and Chetco rivers. Infection among Chetco River chinook has likely resulted from stocking virus-infected fish raised at Elk River Hatchery. The presence of IHN virus in these stocks has resulted in their quarantine to the Elk and Chetco river systems and has prevented the utilization of these salmon to further enhance the fisheries of the southern Oregon coast.

Infectious pancreatic necrosis virus has been isolated from salmonids at only one hatchery site since the epizootics at Fall River and Wizard Falls hatcheries during 1973 and 1975. In 1979, IPN virus was recovered from juvenile steelhead at Warm Springs Hatchery. Presently this is the only known fish rearing facility in the state that has IPN virus infected salmonids.

The destruction of infected stocks followed by strict sanitation procedures was implemented following the epizootics at Fall River and Wizard Falls hatcheries. The success of

such management procedures is demonstrated by the absence of IPN virus in subsequent years among fish reared at these facilities.

The presence of IPN virus among brook trout in the Cascade lakes has not been reevaluated since 1974. These lakes were stocked with trout from Fall River Hatchery prior to 1973 and these fish may have been carriers of IPN virus. Several years of restocking with virus-free brook trout has probably lessened the incidence of IPN virus among trout populations in these lakes (Yamamoto and Kilistoff, 1979).

No further isolations of IPN virus from Columbia River coho salmon have occurred since the original observations during 1971 and 1972. Failure of the virus to cause mortality and become established in these stocks demonstrates that coho salmon are resistant to lethal infection by IPN virus even though they can apparently act as reservoirs (carriers). The replication of IPN virus in coho salmon in the absence of disease has also been reported by Wolf and Pettijohn (1970). The interactions between the virus and host observed in coho salmon infected with IPN virus warrant further investigation.

The elimination of IHN and IPN viruses from fish in certain watersheds is a difficult task. The following control measures have been the most effective means available for this purpose: 1) the use of iodophors for the disinfection of fish eggs and hatchery equipment (Amend and Pietsch, 1972), 2) destruction of infected fish and sanitation of the entire hatchery facility, and 3) the removal of all carrier fish in the vicinity of the hatchery. When one or more of the above procedures are not practical, a quarantine on the movement of fish stocks from hatcheries where viruses are known to occur has proven effective in preventing the spread of both IPN and IHN viruses. Continuous surveillance of salmonid fish stocks and the implementation of aggressive managerial procedures are necessary to restrict the spread of these viruses.

The occurrence of VEN virus among Oregon salmonids has only recently been recognized. Fish from 28 locations have been examined for VEN virus. Thus far the virus has only been observed six times and in three of these cases chum salmon were the host (Table 3). Coho salmon returning to Nehalem Hatchery were also infected with VEN.

Pacific herring from Yaquina and Winchester bays were also found to be heavily infected with VEN virus. The virus obtained from herring is known to infect chum salmon by inoculation and exposure in water (McMillan and Mulcahy, 1979) and must be considered a possible source of virus infection among these and other species of Pacific salmon. The mode of transmission of VEN between marine fish species and salmonids under natural conditions is not clear. This virus is similar to those of the iridovirus group but the discrepancy in diameters of the virus particles observed in erythrocytes of several species of fish make it uncertain whether these represent a single viral entity.

The current interest in the culture of salmon has increased the need for eggs free of viruses. To prevent the introduction or spread of these agents, their occurrence and distribution should be known. This information is required for making appropriate decisions regarding the export or import of fish or fish eggs.

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Isolation and Characterization of a New Reovirus from Chum Salmon

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Abstract

This report describes the isolation of a new virus from adult chum salmon (*Oncorhynchus keta*). The agent was isolated in the CHSE-214 cell line derived from chinook salmon (*Oncorhynchus tschawytscha*) during a required inspection prior to the importation of chum salmon eggs from Japan into the United States. This virus replicates in selected fish cell lines between 10 and 20°C causing a unique cytopathic effect. Physical and chemical studies have shown the virus to be a member of the family Reoviridae and distinct from any known virus of fish. Electron microscopy of negatively stained particles reveals an icosahedral virion approximately 75 nm in diameter composed of a double capsid. Treatment with α-chymotrypsin removed the outer capsid yielding a subviral particle with enhanced infectivity. The virus is chloroform stable indicating the absence of essential lipids. It is resistant to pH 3, and is inactivated at 56°C. Viral replication is not inhibited by 5-fluro-2'-deoxyuridine suggesting an RNA genome and acridine orange stain reveals typical reovirus-like cytoplasmic inclusions. The virus replicates in chum, chinook and kokanee salmon (*Oncorhynchus nerka*) fry and causes focal necrosis in the liver of chum and chinook. No mortality was observed in these three species tested.

Introduction

In October 1978 a viral examination was conducted on a stock of adult chum salmon (*Oncorhynchus keta*) returning to the Tokushibetsu Hatchery, Hokkaido, Japan. This examination was required by Oregon Department of Fish and Wildlife regulations and United States federal law before eggs from these fish could be imported for use by private aquaculture in Oregon.

Samples of ovarian fluid, semen, kidney, and spleen from sexually mature fish were collected and initially prepared in Japan. These samples were sent by air to the Fish Disease Laboratory, Oregon State University Marine Science Center in Newport, Oregon where a diagnostic and certification facility for aquaculture has been in operation since 1977.

During virological examination, an unusual type of cytopathic effect (CPE) was observed in some of the cell cultures. Electron microscopy revealed numerous virus particles in the supernate of infected cultures. This agent appears to be a previously undescribed reovirus of fish and has been tentatively designated chum salmon virus (CSV).

Materials and Methods

Cells and Medium

Monolayer cultures of chinook salmon embryo cells (CHSE-214; Nims, et al., 1970) and rainbow trout gonad (RTG-2; Wolf and Quimby, 1962) were used for the certification examination. The virus was isolated and routinely propagated in the CHSE-214 cell line. The

host range studies were conducted using: CHSE-214, RTG-2, steelhead trout (STE-137; Fryer, et al., 1965), chum salmon heart (CHH-1; Winton and Lannan, unpublished data), kokanee salmon ovary (KO-6; Winton and Lannan, unpublished data), bluegill fry (BF-2; Wolf and Quimby, 1966), brown bullhead (BB; Wolf and Quimby, 1969), fathead minnow (FHM; Gravell and Malsburger, 1965), walleye sarcoma (WC-1; Kelly and Miller, 1978), epithelioma papillosum cyprini (EPC; Tomasec and Fijan, 1971), and largemouth bass (LBF-2; Wolf and Quimby, 1966). All cells were grown in Eagle's minimal essential medium (MEM) supplemented with 10 percent fetal bovine serum (MEM-10) for cell growth and 5 percent serum (MEM-5) for viral replication. Except where noted, cells and virus were grown at 18°C. The methods for the propagation of fish cell lines are described by Wolf and Quimby (1976).

Certification

The examination of adult chum salmon at the Tokushibetsu Hatchery in northern Hokkaido occurred in October of 1978. One hundred fifty semen samples, 150 ovarian fluid samples and 60 kidney and spleen samples were collected from sexually mature animals at the hatchery. Samples were prepared in five-fish pools according to the methods of the American Fisheries Society: Fish Health Section (1975). Briefly, semen and ovarian fluid samples were diluted 1:5 in MEM containing standard tissue culture antibiotics (penicillin, streptomycin, gentamicin and mycostatin). Kidney and spleen pools were homogenized with a mortar and pestle in 20 volumes of Hank's balanced salt solution (BSS). The homogenate was diluted 1:5 in the antibiotic mixture. All tissues and fluids were maintained at refrigeration temperatures (approximately 4°C) during their preparation and transportation. The samples were sent by air to the United States where they were placed on CHSE-214 and RTG-2 cells and incubated at 10 and 18°C.

Electron Microscopy

Supernate from infected CHSE-214 cell cultures was harvested after CPE was complete, centrifuged at 2000 G for 20 minutes to remove cell debris and the virions pelleted by centrifugation at 115,000 G for 1 hour. Virus pellets were resuspended in distilled water and stained for 1 minute with 2 percent phosphotungstic acid (PTA) on Formvar coated grids. Measurement of virus particles used an internal standard of bovine catalase crystals according to the method of Wrigley (1968). All grids were examined in a Phillips 300 electron microscope.

Acridine Orange Stain

Monolayer cultures of CHSE-214 cells were grown on 1.5 cm coverslips and inoculated with 10 $TCID_{50}$ of virus in MEM-5. Controls and infected cells were incubated for 72 hours at 18°C, fixed and stained with acridine orange by the method of Rovozzo (1973), and examined with a fluorescent microscope.

Physical and Chemical Characterization

Heat lability of the virus was measured by incubating a virus suspension at 18, 37, and 56°C. Samples were removed at selected intervals and the titer determined.

Chloroform sensitivity of the virus was determined by the method of Feldman and Wang (1961). One ml of chloroform was added to 2 ml of clarified supernate from an infected cell culture. A control tube of supernate received 1 ml of 0.85 percent NaCl. These mixtures were shaken for 10 minutes. The chloroform treated and control cultures were centrifuged at 600 G for 5 minutes to separate the chloroform in the treated sample and the virus in the aqueous phase and the saline control was titrated.

Resistance to 5-fluoro-2'-deoxyuridine (FUDR) was determined by the method of Rovozzo (1973). Briefly, monolayer cultures of CHSE-214 cells were treated with 10^{-4} M FUDR in BSS. Untreated controls received BSS alone. Treated and untreated cultures were infected with

virus and incubated for 6 hours at 18°C. All cultures were washed with BSS, incubated for seven days in MEM-5 at 18°C and the virus titrated.

The ability of α-chymotrypsin to remove the outer capsid layer of the virion was tested by the method of Joklik (1972). Purified virions in sodium chloride, sodium citrate buffer (SSC, sodium chloride 0.15 M, sodium citrate 0.015 M, pH 7.4) were digested with α-chymotrypsin (100 μg/ml). Treated virions and untreated controls (SSC without chymotrypsin) were incubated for 60 minutes at 37°C and an aliquot removed for titration. The remainder of the incubation mix was pelleted by centrifugation at 115,000 G for 60 minutes. The pellet was resuspended in double distilled water, negatively stained with 3 percent PTA and examined in an electron microscope.

Stability to pH 3 was tested by incubating a suspension of virus at 18°C in MEM adjusted to pH 3. Controls were suspended in MEM at pH 7. After 30 minutes incubation, the virus was titrated.

Virus Replication

The following cell lines were used to test the host range of the virus: CHH-1, CHSE-214, STE-137, KO-6, LBF-2, BF-2, WC-1, EPC, FHM, RTG-2, and BB. Cells were grown in 25 cm^2 flasks in MEM-10 at 18°C until confluent. The growth medium was decanted and 5 ml of MEM-5 containing 200 PFU of virus added to cultures of each cell line. After 14 days incubation at 18°C , 0.1 ml of the supernate from each flask was added to a second set of cultures. After an additional 14 day incubation, the virus produced by each cell line was titered in CHSE-214 cells.

The optimal temperature for virus replication was determined in EPC cells. Monolayer cultures in 75 cm^2 flasks were infected with 500 PFU of virus and 20 ml of MEM-5 added to each flask. The cells were incubated at 5, 10, 15, 20, 25, and 30°C. At 48 hour intervals for 14 days, 1.0 ml of supernate was removed from each flask and the virus titer determined.

In Vivo Studies

The pathogenicity of the virus was tested for chum salmon (*Oncorhynchus keta*), chinook salmon (*Oncorhynchus tschawytscha*), and kokanee salmon (*Oncorhynchus nerka*) fry. Fish weighing 1 to 2 g were injected interperitoneally (i.p.) with 10^4 $TCID_{50}$ of virus in 0.02 ml MEM. Two groups of 20 fish each were injected with virus and two control groups receiving only MEM were held in 10-liter static water aquaria at 12°C for 42 days and observed daily for mortality.

The amount of virus produced by infected chum, chinook and kokanee salmon fry was tested by injecting 1 to 2 g fish i.p. with 10^4 $TCID_{50}$ of virus in 0.02 ml of MEM. At 48 hour intervals for 14 days then at day 21, 28, and 42, three fish were removed, pooled and homogenized in BSS (1:10 w/v) using a Virtis tissue homogenizer. The homogenate was centrifuged at 2000 G for 20 minutes, the supernate filtered through a 0.45 μm filter and the virus titered.

Histological changes in infected chum, chinook and kokanee salmon fry were determined by injecting 1 to 2 g fry with 10^4 $TCID_{50}$ of virus as before. At day 8, 14, 21, 28, 42, two fish were removed and placed in Bouin's fixative. These fish were embedded in parafin and serial 7 μm sections were cut. The mounted sections were stained with hematoxylin and eosin and the internal organs examined by microscope.

Results

Isolation of the Virus

After six days of incubation at 18°C, one of the kidney and spleen pools on CHSE-214 cells began to show several focal areas of CPE. No changes were noted in ovarian fluid or semen samples or in the control cultures. No CPE was observed in any sample placed on RTG-2

cells. These plaque-like areas continued to expand, eventually involving the entire monolayer. When material from the infected cultures was diluted 1:100 in MEM-5 and inoculated onto fresh CHSE-214 cells at 18°C, an identical CPE was observed. A second 1:100 dilution and subculture gave the same results. A third subculture was passed through a 0.22 μm filter and the characteristic plaques were again observed. When infected CHSE-214 cultures were incubated for 72 hours and stained with May-Grünwald-Giemsa stain, the plaque morphology could be seen to involve the destruction of the cell membranes with cytoplasmic fusion leaving intact nuclei at the periphery of the plaque (Figure 1).

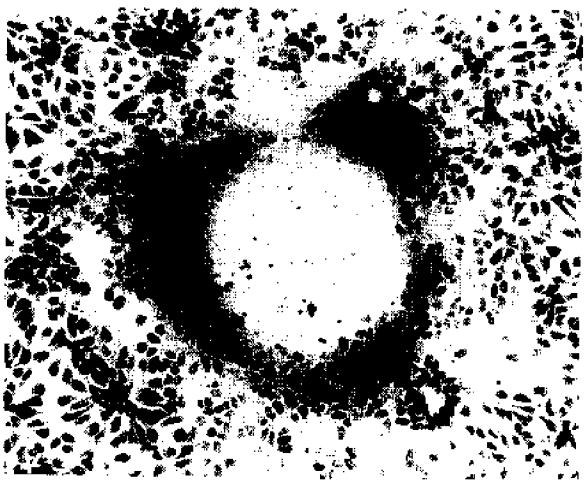

Figure 1. Cytopathic effect produced by chum salmon virus in CHSE-214 cells incubated at 18°C for 72 hours. May-Grünwald-Giemsa stain.

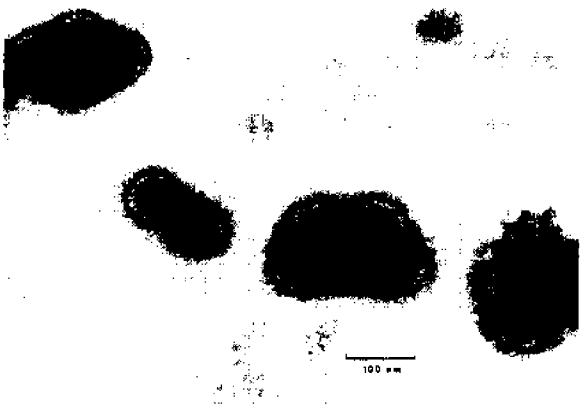

Figure 2. Electron micrograph of chum salmon virus.

Electron Microscopy

Negatively stained virions revealed particles with icosahedral symmetry and a double capsid structure (Figure 2). The particles are 75 nm in diameter and both complete and coreless virions can be seen. Twenty peripheral capsomeres are present and no envelope was

detected. These morphological features are characteristic of viruses of the Reoviridae (Joklik, 1974).

Acridine Orange Stains

Control cultures were normal with yellow-green nuclei and red staining cytoplasm. Infected cultures demonstrated plaques with fused cytoplasm containing green inclusion bodies. The nuclei appeared normal. These green cytoplasmic inclusions are presumptive evidence of double stranded nucleic acid and are usually observed in cells infected with reoviruses.

Physical Characterization

The virus was stable for 24 hours at 18 and 37°C, but rapidly inactivated at 56°C. The original suspension containing 2.7×10^5 PFU/ml declined in titer to 6.8×10^3 PFU/ml in one hour and no infectious virus remained after six hours incubation at 56°C. Loss of 1 log or greater in one hour is indicative of heat lability (Rovozzo, 1973).

Chloroform treatment did not reduce infectivity of the virus. The NaCl treated control had a titer of 5.9×10^5 PFU/ml while the chloroform treated suspension gave a titer of 1.6×10^6 PFU/ml. This resistance to chloroform is evidence that the virus lacks an essential lipid containing envelope.

The deoxyuridine analog FUDR was not effective in blocking viral replication. Control cultures incubated without FUDR reached a titer of 1.8×10^5 PFU/ml while FUDR treated cultures had a titer of 1.2×10^5 PFU/ml. The lack of inhibition by halogenated pyrimidines is evidence that the virus possesses an RNA genome.

Electron micrographs of virions treated with α-chymotrypsin demonstrated the outer capsid layer of the virion had been removed leaving a subviral particle of 50 to 55 nm in diameter. The titer of the untreated control was 5.0×10^7 $TCID_{50}$/ml while chymotrypsin treated virus had a titer of 6.3×10^8 $TCID_{50}$/ml. This increase in viral titer is probably due to the enhanced infectivity associated with the removal of the outer capsid layer as observed in certain types of reoviruses (Spendlove, et al., 1970).

This virus was not inactivated by exposure to pH 3. The control titer at pH 7 was 3.2×10^5 $TCID_{50}$/ml and the pH 3 titer was 5.0×10^6 $TCID_{50}$/ml. The increase in virus titer seen after pH 3 treatment is also likely due to the removal of the outer capsid layer.

Virus Replication

With the exception of the RTG-2 cell line, the best virus replication occurred in cells derived from salmonid fish (Table 1). The RTG-2, FHM and BB cell lines showed no cytopathic effect and produced low levels of virus. Other non-salmonid lines exhibited various degrees of CPE and produced moderate virus titers.

Table 1. Replication of chum salmon virus in selected fish cell lines.

Cell line	Abbreviation	Titer $TCID_{50}$/ml
Chum salmon	CHH-1	2.5×10^8
Chinook salmon	CHSE-214	1.6×10^6
Steelhead trout	STE-137	6.3×10^5
Kokanee salmon	KO-6	4.0×10^5
Largemouth bass	LBF-2	1.6×10^5
Bluegill	BF-2	6.3×10^4
Walleye	WC-1	6.3×10^4
Carp	EPC	3.9×10^4
Fathead minnow	FHM	5.0×10^2
Rainbow trout	RTG-2	5.0×10^2
Brown bullhead	BB	4.0×10^2

The optimal temperature for viral replication was determined to be 15 to 20°C (Figure 3). The EPC cell line was used for these experiments as it tolerates a higher temperature than the salmonid lines. The extent of CPE correlated with the virus titer produced at each temperature. At 10°C, viral replication was slower and CPE less extensive than at 15 or 20°C.

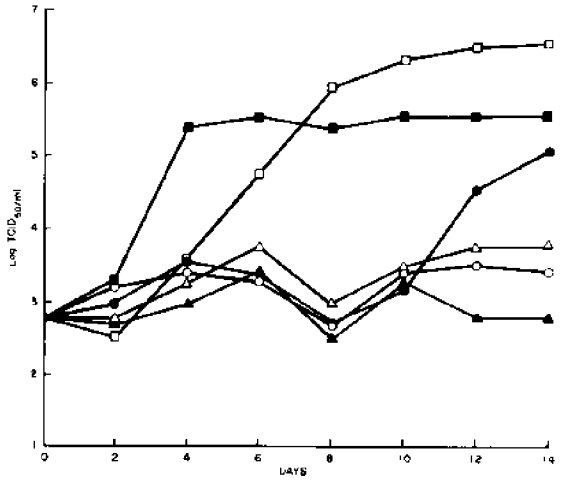

Figure 3. Replication of chum salmon virus in EPC cells at selected temperatures. Symbols: 05°C; ● 10°C; □ 15°C; ■ 20°C; △ 25°C; ▲ 30°C.

In Vivo Studies

No significant mortality occurred in the chum, chinook, or kokanee salmon fry injected with the virus and no gross external signs were seen in fry held for 42 days. For chinook, two of 40 control fish and none of 40 virus injected fish died during the experiment. Among the kokanee, no control and three of 40 experimental fish died. In the chum salmon fry, no control and two of 40 virus injected fish died in the 42 day period.

The virus replicates in all three species tested (Figure 4). Virus titers increased slowly in chinook and actively in chum and kokanee salmon fry. Maximum titers in chum salmon occurred at 21 days after injection and were significantly above the titers of the other species. At the end of the 42 day period, all three species had similar titers.

Histological examination provided evidence of pathology in chum and chinook fry. Numerous areas of focal necrosis were observed in the liver of infected fish. These lesions began as small areas of necrosis at day 8. By day 14, the lesions were maximal in size and number in the chum salmon fry (Figure 5). At day 21 and after, the lesions began to heal and normal liver cellular architecture began to return. A similar histological picture was seen in chinook fry except that the extent and severity of the lesions was less. No pathology was observed in the liver of infected kokanee salmon. Other organs of all three species were unremarkable.

Discussion

The agent reported here and termed "chum salmon virus" or CSV appears to be a previously undescribed virus of fish. The virus shares morphological and chemical features with the Reoviridae. Like all members of the family, it has icosahedral symmetry and a double stranded RNA genome. The lack of an envelope, stability to pH 3, 75 nm size, and creation of subviral particles after treatment with chymotrypsin, suggest this virus is closely related to the genus *Orthoreovirus*. This genus is composed primarily of viruses isolated from

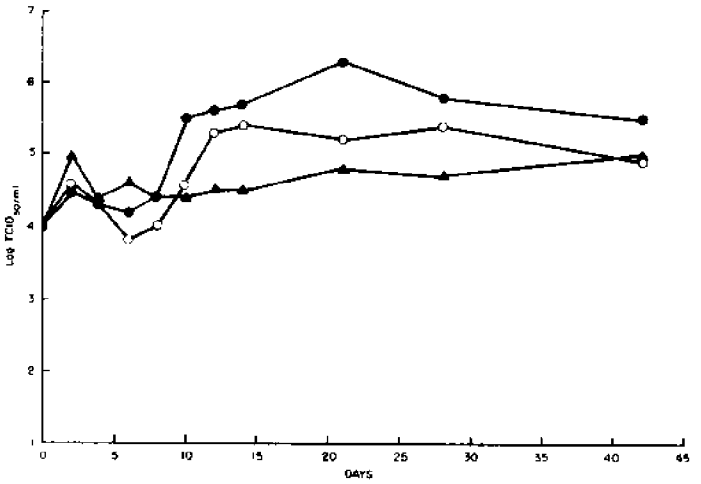

Figure 4. Replication of chum salmon virus in chum, chinook and kokanee salmon fry held for 42 days in 12°C aquaria. Symbols: ● chum; ○ kokanee; ▲ chinook.

Figure 5. Focal necrotic lesion in the kidney of chum salmon injected with chum salmon virus and held for 14 days in 12°C aquaria.

homeothermic vertebrates. The virus described here lacks the typical *Orthoreovirus* stability to 56°C. The temperature optimum of 15 to 20°C indicates this is a virus of poikilotherms.

The origin of this virus is unclear. While viral replication has been demonstrated in the three species of salmonid fish tested, the agent may have its normal host among other poikilothermic vertebrates or invertebrates producing disease in those animals. No report of the isolation of a similar virus is mentioned in a review of the virus diseases of fish in Japan (Sano, 1976). A recent listing by Wolf and Mann (1980) also contains no reference to the isolation of a true reovirus from fish. Meyers (1979) describes a virus isolated from American oysters (*Crassostrea virginica*) in Long Island Sound, New York, with properties similar to the agent described here. The host range studies and certain chemical features of the Meyers isolate suggest it is a closely related, but not identical, virus. Further testing will be necessary to establish the taxonomic relationship of these two agents.

The discovery of a previously unknown viral agent in a population of fish selected for im-

portation to a new geographic area demonstrates the need for continued surveillance of salmonid stocks and the necessity for controlling the spread of untreatable diseases through certification programs. While the pathogenicity of this virus seems low for the species tested, any viral disease causing focal necrosis of the liver may weaken the resistance of infected fish to other biological or environmental insult. The movement of virus infected stocks of fish into locations where the virus is not endemic may expose native stocks of fish to a virus for which they have little natural resistance acquired through long exposure and genetic selection. Virus infected stocks of fish are undesirable for use in aquaculture as the stress associated with normal culture practices will often result in large mortality among these fish.

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Section X
Other

Proc. No. Pac. Aquaculture Symp.,
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International Law Problems of Salmon Fisheries Management

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Among the factors impacting the development of salmon fisheries in the North Pacific, the international law fisheries management measures play a significant role. During the past decades principles of anadromous fish management have not virtually changed.

Up to the present time the salmon stocks management has been exercised mostly through bilateral agreements between the state of origin of anadromous fish and a state engaged in high seas fishing for salmon. Despite the obvious advantages of bilateral agreements, such as specificity, direct impact on economic relations of two countries, etc., they fail to provide the realistic basis for rational management of fisheries. As a result of heavy high seas fishing the salmon stocks remain in a depressed condition.

Establishment of 200-mile economic and fishing zones by the majority of coastal states has not resolved the problem of rebuilding the salmon stocks for the states of origin because measures on regulation of high seas fishing under bilateral agreements at present appear to be ineffective.

In this connection the necessity of a multilateral approach to the salmon management problem is quite evident. This has been clearly shown in the fisheries sessions of the Third Law of the Sea Conference and in the Protocol of the First International Conference on Pacific Salmon. As an alternate solution to this problem the authors suggest moving the foreign fishing effort from the high seas to the coastal areas of a state of origin. This would eliminate all possible shortcomings of the high seas fisheries, since stronger restrictive measures by the state of origin could be imposed within its own 200-mile zone.

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