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PROGRESS in

SPORT FISHERY RESEARCH, 1957-1958

UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE
BUREAU OF SPORT FISHERIES AND WILDLIFE
CIRCULAR 57

Cover:--Abrams Falls, Great Smoky Mountains National Park. The reclamation of lower Abrams Creek was initiated at the Falls on June 8, 1957. Spectators were waiting to collect rainbow trout, carp, redhorse suckers, sunfish, rock bass, and smallmouth bass.

Photo by Eugene W. Surber, Branch of Federal Aid, Bureau of Sport Fisheries and Wildlife, Atlanta.

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PROGRESS IN SPORT FISHERY RESEARCH, 1957–58

Compiled in the Branch of Fishery Research

U. S. FISH AND WILDLIFE SERVICE

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CONTENTS

Henrietta M. Mugmon
Compositor

INTRODUCTION

TWENTY YEARS AGO, in the introduction to "Progress in Biological Inquiries", an administrative report of the Bureau of Fisheries, Mr. Elmer Higgins said,

> "The underlying philosophy of fishery conservation based on a continually growing body of scientific knowledge is now so well understood and the program of investigation under way during the past decade has justified itself so completely, both in its theoretical aspects and its practical applications, that it has acquired a momentum that in itself is a protection against irresponsible diversion of interest or activity into byways of local application or temporary value."

In the years since 1938, there has been many a change in the organization, direction and scope of fishery biological research. "Aquicultural investigations", as sport fishery research was called then, had an annual appropriation of about $42,000. Dr. H. S. Davis ("Care and Diseases of Trout") was in charge. The "meager amount of funds for publication" of research findings was only 4.7 percent of the appropriation, but Dr. Davis and his colleagues - F. F. Fish, Paul Needham, Osgood Smith, Eugene Surber - published on stream and lake surveys, trout stream management, fish disease treatment, and bass parasites. Russell Lord, in Vermont, was studying angler harvest and evaluating trout stocking as a means of maintaining stable fish populations on three streams and a lake set aside for the purpose by the State. Tom Chamberlain was continuing a program of experimental fish management in Pisgah National Forest in North Carolina. Research on the vitamin requirements of trout, using synthetic diets and the new vitamin concentrates was just getting underway at the Cortland laboratory; Dr. C. M. McCay of Cornell, A. V. Tunison of New York State, and Dr. A. M. Phillips, Jr., of the Bureau of Fisheries were the cooperating staff members. Dr. Stillman Wright was working with the Forest Service and the several States in the intermountain region on problems of fish management, principally on Fish and Bear Lakes in Utah. A new warmwater fish laboratory was opened at Welaka, Florida, where Dr. O. L. Meehean began studies of bass production and propagation. Dr. James Gutsell, at Leetown, was carrying on feeding experiments with dry meals to determine their effect on growth, mortality and egg production. Francis Sumner completed a study of biological and physical conditions of Pyramid Lake, Nevada, concluding that rehabilitation by any means was doubtful.

The war which followed soon after, broke up the staff and to a large extent the continuity of their researches. Postwar "reconstruction" led to some new lines of research, resumption of others, and abandonment of still others. In general, however, sport fishery research of the Fish and Wildlife Service was still concentrated on problems of fish culture and fish management; pollution and water quality research was dropped when this responsibility was transferred to the U. S. Public Health Service.

The reorganization of the Fish and Wildlife Service, following passage of legislation in 1956, took all commercial fishery activities into a new Bureau of Commercial Fisheries, including, by administrative decision, all marine fishery research. Some marine and anadromous species of both sport and commercial importance, like shad, striped bass and salmon, are the concern of that Bureau. Research in the field of salmon culture, however, is a responsibility of the Bureau of Sport Fisheries and Wildlife.

In the 18 months since establishment of the Branch of Fishery Research there have been but few substantive changes. Sport fishery research, highly decentralized but not regionalized, is concerned with fish propagation research: trout and salmon culture, and warmwater fish propagation;

and fishery management research: on public waters, like those of the National Park Service which we have used as outdoor laboratories for studies of lake and stream productivity and experimental management techniques; and research on environmental influences which affect the welfare of the fish.

We do not yet pretend to have a well-balanced research program. We recognize some very large unfilled needs: -- fundamental research on the productivity of large reservoirs to find and state the principles necessary to their understanding and management; -- warmwater fish research for the improvement of propagation, to fill gaps in our knowledge about their nutrition, their diseases, and the best methods for environment manipulation to get the best results from stocking; -- finding criteria by which we can recognize the hatchery fish having the highest survival potential; --research in the field of genetics and selective breeding for desirable qualities of growth, disease resistance, and fertility; -- continuous study, testing, evaluation and development of fish-cultural techniques; -- research on the physiological responses of fish to understand their behaviour and to apply this knowledge; -- studies leading to improvement of fish distribution techniques and equipment; -- marine sport fish research, recognizing the rapid growth of salt water angling and the concurrent threats from vast developmental and engineering projects which are changing the environment of many of the finest gamefish species; -- research on the short- and long-range effects of pesticides and radioactive waste disposal inland; -- research, or support of research, in fish taxonomy and systematics.

As this report is being compiled, there are good prospects for Bureau research in some of these fields. We hope the report for the year ending December 31, 1959 will reflect progress in meeting more needs for knowledge than this one.

Although several of the reports following this introduction suggest it, I want to mention the enormously valuable cooperation with persons, agencies and institutions outside of the Bureau, without which much of the research progress would not have been possible. The Bureau of Commercial Fisheries, National Park Service, Forest Service, and United States Public Health Service have contributed much in facilities, assistance and collaboration. The State agencies of Maine, New York, Colorado, Montana, California, Oregon, Washington and Nevada have been helpful in many ways. Cornell University, Utah State University, the University of Washington, and Colorado State University have provided student assistants, consultative services, entered into contractual arrangements for research or otherwise participated.

Within the Bureau of Sport Fisheries and Wildlife, excellent arrangements have been continued with three co-sponsored in-service training schools for fish culturists at the Cortland, Leetown and Seattle laboratories of the Branch of Fishery Research. The Branch of Fish Hatcheries has screened applications, completed arrangements for attendance and given assistance in a variety of ways. The three branches of the Division of Sport Fisheries -- Fishery Research, Fish Hatcheries, and Fishery Management Services -- have edited and produced the Progressive Fish-Culturist, a quarterly periodical for fishery biologists and fish-culturists which began publication 25 years ago and now has an international circulation.

FISH MANAGEMENT RESEARCH

Appalachian Sport Fishery Investigations
Leetown (P. O. Kearneysville), W. Va.
Robert E. Lennon, Chief

Streamside toxicity experiments with 23 species of fish were completed in Great Smoky Mountains National Park.

Surveys and creel census operations have shown that the reclamations of Indian Creek and Abrams Creek were successful.

The sport-fishing-only program in Great Smoky Mountains National Park was expanded to permit the possession of trophy fish and to include year-round fishing.

Appalachian-strain brook trout have been used successfully in restoration experiments.

Surveys were made on 36 streams in Great Smoky Mountains National Park and 30 streams in Shenandoah National Park in 1958.

Applications of resistivity measurements in fishery investigations were defined.

Experiments in the improvement of electrofishing with stock salt were completed.

Electrofishing trials were observed in 7 States and 3 national parks.

GREAT SMOKY MOUNTAINS NATIONAL PARK

Stream reclamation

The widespread success of attempts to improve sport fishing through the reclamation of lakes and ponds with toxicants has drawn attention of fishery workers to the possibilities of reclaiming streams. Since there are many opportunities to restore native species and to improve sport fishing on southeast streams, experiments in the reclamation of running waters were conducted in 1957 and 1958.

These experiments have shown that there is a factor of utmost importance in the reclamation of running waters which seldom has to be considered in the reclamation of lakes. In a stream, the duration of exposure to a toxicant is as important as an adequate concentration to effect the desired kill of fish. If either factor is less than sufficient, fish will survive. In addition, the inverse relationship between duration of exposure and concentration is complicated by the stretch-out and dilution of toxicant over miles of stream and by the chemistry and temperature of the water.

Little information is available on the duration of exposure necessary to kill fish with various concentrations of rotenone. Tests with a commercial preparation were made, therefore, in streamside troughs on Indian Creek and Abrams Creek prior to the reclamation of these streams. They showed that 1 ppm of toxicant, maintained for a minimum of 1 hour, was sufficient to eliminate rainbow trout and longnose dace in Indian Creek. A similar concentration, but maintained in an uninterrupted bolt for 6 hours, was required to kill carp and less resistant species in Abrams Creek. Additional toxicity tests were made with two commercial rotenone preparations in May, June, and December, 1958, to provide essential data for the probable reclamation of other streams. Four-hundred-sixty-one fish of 23 species were exposed to several concentrations of the toxicants for varying periods of time and at various temperatures in abandoned rearing pools on Anthony Creek, a tributary of Abrams Creek. The wild goldfish, blue catfish, brown bullhead, and some of the carp used in the tests were furnished by the Tennessee Game and Fish Commission. Other species, with the exception

of hatchery-reared brown trout, rainbow trout, and eastern brook trout, were collected in park streams.

Some carp, some bullheads, and all goldfish survived 5- to 6-hour exposure to 0.5-ppm concentrations at 60 to 64 degrees F. The other species, including trout, suckers, dace, shiners, stoneroller, darters, smallmouth bass, rock bass, sunfishes, and freshwater sculpins were killed. Longer durations of exposure or stronger concentrations were required for kills at 50° F. and below. Carp, goldfish, and some stonerollers, however, survived 5.5-hour exposures to 2 ppm at 40° F. Indeed, 4 goldfish survived a 77-hour exposure to 2 ppm at 40° F.

Only goldfish survived 5-hour exposures to 4-ppm concentrations of the two commercial products at 65° and 49° respectively. It appears that the reclamation of a large stream which contains goldfish might be impractical due to the amount of toxicant required to maintain a long duration of exposure. The need for selective or improved toxicants is obvious.

The tests demonstrated that rainbow trout, suckers, carp, stonerollers, smallmouth bass, rock bass, and sunfishes which were immobilized in a killing concentration of rotenone for a sub-lethal period of time would recover when removed to fresh water. It was concluded, however, that a concentration of 1 ppm, which was maintained in an uninterrupted bolt for 6 hours, in water of 60° F. or over, would kill all the species of stream fish tested with the exception of goldfish and possibly brown bullheads.

Indian Creek

Unsuccessful attempts were made about 20 years ago to restore brook trout in Indian Creek through heavy stocking. The failures were presumed to be due to competition from the established rainbow trout. A 6.5-mile section of the stream was reclaimed in May 1957 and stocked with 2.5-inch, native-strain brook trout in the following month. Subsequent surveys through 1957 and 1958 showed that these trout maintained good growth and condition. Some attained legal size (7 inches) by the following spring. Specimens up to 8.3 inches long were checked in anglers' creels shortly after the opening of the 1958 fishing season.

The surveys also showed that some rainbow trout fry had survived the reclamation process since small numbers of young-of-the-year specimens were collected in the summer and fall of 1957. No other rainbows have been taken, however. The stream was flowing at more than twice the normal volume on the date of reclamation. It is assumed that this unfortunate circumstance contributed to the escape of some of the fry which were 0.9 to 1.2 inches long at the time.

Abrams Creek

The lower 14.6 miles of Abrams Creek was reclaimed in June 1957 in cooperation with an effort by the Tennessee Game and Fish Commission to reclaim that portion of the Little Tennessee River which would be inundated by the new Chilhowee Dam. The State's objective was to establish a trout fishery in the new lake. It was necessary, therefore, to eradicate the abundant rough fishes inhabiting the river and lower Abrams Creek.

The technique for the administration of rotenone in adequate amounts for an adequate period of time proved correct. It was introduced in the stream at the rate of 5 ppm for the first hour and 1 ppm for 5 hours thereafter. The bolt was repeatedly strengthened with 1 ppm applications at pre-determined stations downstream. Forty-seven species of fish were eliminated from Abrams Creek and tributaries. Observations through the summer and fall failed to disclose any survivors.

Twenty-four-hundred catchable size rainbow trout were stocked in the stream soon after the reclamation to provide summer fishing. A total of 30,800 fingerling rainbows was stocked in the fall. Their survival and growth were exceptionally good. The average length of trout in creels during the first week of the 1958 fishing season was 10.6 inches (range: 8.0 to 13.8 inches). Limit catches were the rule.

Abundant spawning occurred in the re-

claimed section of Abrams Creek between March 5 and 12, 1958, despite the fact that most of the fish were yearlings. Viable ova were found in the redds and an excellent hatch took place during the last 10 days of April. Young-of-the-year fish were numerous in the fall and plans to stock additional fingerling trout have been postponed indefinitely.

The results obtained in the reclamation of Indian Creek and Abrams Creek demonstrate conclusively that populations of preferred game fish can be restored or established quickly and practically in streams by eliminating rough and competitive species. Reclamation operations on streams should be scheduled, if possible, to take advantage of the following situations: low water levels, the warmest water temperatures, and after all spawning for the season is well past.

Experimental regulation streams

The popularity of the sport-fishing-only program on Bradley Fork and West Prong Little Pigeon River led to its expansion in 1958. On May 16, the provision that all trout be returned to the water unharmed was relaxed to permit possession of trophy fish over 16 inches long. Beginning on September 1, the two streams were opened to year-round fishing. In addition a 15-mile section of lower Little River and a 6-mile section of Oconaluftee River were opened to fishing under sport-fishing-only regulations from September 1 through May 15. These sections will remain open to fishing under general regulations from May 16 through August 31.

The National Park Service has cooperated splendidly in the expanded program. The year-round fishing on the 4 streams will increase recreational opportunities for spring and fall guests in the park.

Estimates of fish populations in the special-regulation streams were made in September and October. Bradley Fork had 56 pounds of rainbow trout per acre. The West Prong Little Pigeon had 25 pounds of rainbow trout per acre in the lower section and 19 pounds of brook trout per acre in the upper section. The lower section of Little River had 4 pounds of rainbow trout and 26 pounds of smallmouth bass per acre. Oconaluftee River had 53 pounds of rainbow trout per acre.

It has been estimated on the basis of periodic checks that there have been 498 fishermen on Bradley Fork, 121 on West Prong Little Pigeon, 394 on lower Little River, and 114 on Oconaluftee River between September 1 and November 30. This amount of use is considered good in view of the fact that very little publicity was given to the extension of the open season.

It is difficult to measure angler success on sport-fishing-only streams. Some reliable results were obtained from 5 fishermen of various abilities who made a total of 8 trips on Bradley Fork and 8 on the West Prong Little Pigeon during September and October. The total catches per angler on Bradley ranged from 7 to 57 trout. The fish ranged from 3.5 to 10.3 inches long. The rates of catch of trout 7 or more inches long ranged from 0.9 to 11.4 fish per hour.

The total catches per fisherman on West Prong Little Pigeon ranged also from 7 to 57 trout. The range in length for brook trout and rainbow trout was 3.0 to 13.4 inches. The rates of catch of trout 7 or more inches long ranged from 0.7 to 13.0 fish per hour.

Trophy-size trout were observed in both streams and some were reported caught. Most of the fishermen contacted volunteered opinions that the fall fishing was excellent.

The restoration of eastern brook trout

Experiments in the restoration of brook trout in several Park streams demonstrate the advantage of using the native strain. Plantings of Appalachian-strain brook trout have succeeded where northern-strain fish failed to become established. Fingerlings of the newly domesticated Appalachian-strain have been furnished generously by the U. S. Fish-Cultural Station at Walhalla, S. C.

The first experiment in restoration was made on the West Prong Little Pigeon. The upper 6 miles of the stream were devoid of fish follow-

ing a disastrous washout in 1951. Fingerling-size, Appalachian brook trout were stocked in 1954 and each year thereafter. Despite the low productivity potential of the stream, high quality brook trout up to 14 inches long have afforded excellent fishing since 1955 under sport-fishing-only regulations. In addition, as a mature population developed, natural reproduction has increased. Essentially the same results have been obtained on Road Prong and Alum Cave Prong.

Appalachian-strain fish have been packed in and stocked in near-barren headwaters of Ramsey Prong and Porters Creek since 1956. Fingerlings stocked in June at 2.5 inches long have been entering the legal catch (7 or more inches long) in the following season. The only failure resulted when fingerlings were stocked in Cosby Creek, amid heavy populations of cyprinids.

The restoration of brook trout is important management-wise since many headwater streams in the Smokies are too acid for rainbow trout but are suitable for brook trout. Native populations of brook trout in some of these waters were wiped out by logging, floods, fireash, and poaching in pre-Park days. The re-establishment of brook trout fisheries in such waters appears possible and worthwhile.

Surveys of trout populations

Most of the major watersheds in Great Smoky Mountains National Park were surveyed in the fall seasons of 1957 and 1958. Of 31 streams sampled in the fall of 1958, 11 had trout populations which exceeded 50 pounds per acre; 13 streams had populations which ranged from 25 pounds to 49 pounds per acre; and 7 streams had less than 25 pounds of trout per acre. Included are rainbow trout, brook trout, and brown trout, of which the rainbow is the most abundant. Populations of smallmouth bass and largemouth bass were observed in the lower courses of some streams.

Young-of-the-year rainbow trout were particularly numerous in 1958. The previous peak in 0-year fish took place in 1954 and this strong year-class contributed to a high proportion of legal-size fish in the streams in 1956. Also, creel census data show that the rate of catch per angler was higher in 1956 than in the immediately preceding and following years. If there is a 4-year cycle for rainbow trout in Park streams, another peak in numbers of legal-size fish should occur in 1960.

An analysis of the age and growth of rainbow trout in several streams indicates that the most rapid growth occurs during their third and fourth summers. Life is short in these streams and few rainbows survive longer than 4 years. Less than 0.1 percent of the tens of thousands of trout collected were 5 years of age or older. The relatively few 5- to 7-year old specimens taken ranged from 13.4 to 20.8 inches long.

The spawning success of rainbow trout is very small on the lower courses of such beautiful and important streams as Little River and Little Pigeon River. This situation was observed year after year despite the fact that good numbers of mature trout were present. The possible limiting factor was observed on Little River in March 1958. Groups of rainbow trout redds which contained numerous and viable ova were placed under daily observation. Before hatching time in April, swarms of stonerollers began spawning activities in the same beds that the trout used. All traces of the trout redds were destroyed and no ova could then be found. Only one trout fry was subsequently observed in the vicinity of a bed which contained several large redds.

There are very large populations of stonerollers in the lower sections of most Park streams. Their competition for spawning grounds might well limit the reproduction of rainbow trout.

SHENANDOAH NATIONAL PARK

Surveys of trout populations

Thirty of the principal streams in the Park were surveyed with electrofishing gear in the fall of 1957 and in the spring of 1958. Native populations of brook trout were found in all but 6 of the streams. Estimates of the populations ranged as high as 65 pounds per acre.

A striking and unfortunate feature of the gamefish resource in the Park is its strong reflection of the weather cycle. In general, the streams rank as good to excellent for native trout in "normal" and wet years. Many, however, cease to be trout streams during dry and drought-marked years. In this respect, therefore, the streams must be considered marginal for trout. Cognizance of this situation is essential to the formulation of any short-term or long-term management programs for the fishery.

OTHER

Applications of a resistivity meter

The electrical resistivity of water can be quickly and easily measured in the field with a portable resistivity meter. Experiments conducted in the laboratory and on streams demonstrated that the relationship between resistivity and electrolytes in water can be exploited in several valuable ways in fishery investigations. Applications of a resistivity meter include the following:

1. Measurements of resistivity can be used to estimate the contents of total dissolved solids in non-turbid, freshwaters. Thus the long and tedious evaporation technique is avoided.

2. A measurement of resistivity indicates the softness or hardness of water on a relative scale (table 1).

3. The meter can be used to detect known amounts of common salt in a stream. Accurate determinations can thereby be made of the velocity, stretch-out, and dilution of a solute in running water. These determinations can be made over miles of a stream under weather and water conditions which would preclude the use of marker dyes. The results of such observations were utilized in the reclamation of Indian Creek and Abrams Creek.

4. The design and selection of electrofishing gear must be related to measurements of resistivity to achieve efficiency in operation. The resistivity of the water dictates the type of electrode system needed, the proper spacing of electrodes, the type of power and voltage level, and whether or not salt is needed to reduce the resistivity to a workable level.

Application of salt in electrofishing

Blocks of stock salt provide an effective and economical means of reducing high resistivities and improving electrofishing in soft water. One or 2, 50-pound blocks were usually sufficient in 28 test streams to reduce resistivities from a maximum of 207,000 ohms to a range of 25,000 to 50,000 ohms. A block lasts up to 4 hours in running water.

Among the improvements noted in electrofishing in salted water were the following: The effective range of the electrode system was increased; a greater proportion of available fish were taken on initial passes through test areas; larger numbers of fish were taken in salted waters than in unsalted waters; and fish were more thoroughly stunned and easier to capture.

Extensive trials have shown that the use of stock salt in conjunction with alternate-polarity, electrofishing gear provides the best means for all-season sampling of fish populations in the extremely high resistivity streams of the southern Appalachian region.

Demonstrations of equipment and techniques of electrofishing

We participated with State and Federal fishery biologists in electrofishing trials on Atlantic salmon streams in Maine, on trout streams in New Hampshire, on warmwater lakes in New York, on trout streams in New Jersey, on bass and trout streams in West Virginia, on bass streams in Virginia, and on trout and bass streams in Tennessee. Organized trials were also conducted on brook trout streams in Shenandoah National Park, on rainbow trout streams in Great Smoky Mountains National Park, and on rainbow trout and cutthroat trout streams in Yellowstone National Park. AC and DC, hand-operated and boat-mounted systems were tested. The test waters ranged from hard to extremely soft, with resistivities of 2,300 to 207,000 ohms cm^3.

The demonstrations provided a valuable opportunity to gather a wide range of data on the performance of electrofishing gear in waters of greatly different resistivities and on a variety of

Table 1:- Types of fresh water classified according to total hardness with approximate ranges of conductivity and resistivity for each type.

Type of water	Total hardness ($CaCO_3$ in ppm)	Conductivity (micromhos at 77°F.)	Resistivity (ohms at 77°F.)
Very soft	4 - 14	14 - 58	71,400 - 17,000
Soft	15 - 49	60 -124	16,600 - 8,000
Medium hard	50 - 99	130 -224	7,700 - 4,300
Hard	100 -199	235 -895	4,200 - 1,100
Very hard	over 200	over 900	less than 1,000

cold water and warmwater fishes. In each trial, the results obtained with a certain type of gear were related to measurements of resistivity. Analyses of these trials made clear the absolute necessity of tailoring the equipment and techniques of electrofishing to the resistivities of the waters. A rough guide for the selection of appropriate electrode systems and power supplies is presented below.

Resistivity (ohms)	Appropriate electrofishing gear
2,000- 5,000	115 V., AC; paddle-type electrodes 115 V., DC; straight or pulsed DC gear 230 V., DC; straight or pulsed DC gear
5,000- 10,000	230 V., AC; paddle-type electrodes 230 V., AC; alternate-polarity electrodes 230 V., DC; straight or pulsed DC gear
10,000- 20,000	230 V., AC; alternate-polarity electrodes 230 V., DC; pulsed DC gear
20,000- 50,000	230 V., AC; alternate-polarity electrodes 230 V., DC; variable-pulse with special electrodes
50,000-200,000	230 V., AC; alternate-polarity electrodes, with salt.

In preparation

Lennon, Robert E., and Phillip S. Parker
The reclamation of Indian and Abrams Creeks in Great Smoky Mountains National Park.

Atlantic Salmon Investigations
Boothbay Harbor, Maine
Alden P. Stickney, Chief

1957

The final tally of adult salmon was as follows (all fish unmarked):

Validated rod catch (not included in weir count)	13
Weir count	56

The weir was taken down for winter on November 15.

Stomach analyses of eels were negative for salmon parr. One parr was found in a pickerel stomach.

Hatchery fingerlings were planted October 11-12 at two sites, each lot differently marked. Average length of pooled sample from both lots was 83 mm. (3-1/4 inches).

1958

The installation of weir in spring was delayed by high water and log drive. Most of smolt migration was lost, although one-year-old group planted the previous fall was probably adequately counted.

Tally of one-year-olds was 165: 97 of planted lot A and 71 of planted lot B. Average size of random samples: 144 mm. for lot A and 137 mm. for lot B. These differences were found not to be statistically significant.

The count of upstream migrants was 6 grilse, 4 four-year-olds, and 1 five-year-old up until the weir was breached on October 1.

Experiments in holding smolts for extended rearing in estuarine impoundment found impracticable, although holding them in screen live cars showed promise. Smolts held in aquarium and fed live fish grew well during summer.

Lennon and Parker from the Appalachian Sport Fishery Investigations spent several days on the Sheepscot demonstrating electrofishing gear.

The weir was overhauled during the low water period, and a few changes in design made to facilitate early installation in spring.

An experiment in laboratory tanks failed to demonstrate any effect of dissolved iodides in the water on the smoltification of parr or their ability to withstand salt water.

The hatchery fish for planting in the Sheepscot were graded into size groups and differentially marked to see what relation exists between size at planting and precocious migration of one-year-olds. These fish were planted on October 16-17 in two localities, one above Coopers Mills where no wild salmon parr are present.

Closer liaison seems to be developing with local sportsmen's groups.

Although encouraging progress has been made in recent years towards the restoration of Atlantic salmon in Maine rivers, it has become increasingly apparent that the contribution to these runs from planted hatchery stock has not been nearly as great as would be desirable. Very few adult salmon of hatchery origin have ever been taken by anglers, and in streams where fish can be individually checked as they enter the percentage of marked (hatchery) salmon has been very low.

Realizing the potential value of hatchery production to implement the management of sport fish populations, it has been one of the aims of the Atlantic Salmon Investigations to seek the causes of the poor survival of hatchery reared salmon and to devise methods for preventing or circumventing these losses. To achieve these ends the problem has been attacked from three angles:

1. To determine the extent of river and of sea mortality by means of a counting weir installed in a river where salmon are stocked.

2. To study the possible causes of mortality, particularly predation.

3. To experiment with methods for decreasing mortality.

In addition to these major goals the work of the Atlantic Salmon Investigations has been to make observations on the growth, food habits, physiology and other aspects of the biology of the salmon; to cooperate with State agencies engaged in Atlantic salmon conservation; and to make general ecological studies in the fluvial, estuarine and coastal marine habitats which might be of importance in understanding more clearly the life history of the salmon.

Operation of the counting weir in the Sheepscot River

This weir has not been eminently successful. Built in 1956 as a temporary structure in the hope that eventually it could be replaced with a permanent year-round installation, it has withstood severe conditions remarkably well. However, heavy freshets with their associated debris have, on several occasions, wrought just enough damage to render some of the counts of fish unreliable. Furthermore, unless conditions are extremely favorable, the weir cannot be installed early enough in the spring to be certain of catching the early part of the smolt migration.

The 1957 season was fairly successful. Slight damage was incurred during one freshet in the spring and some smolts escaped uncounted, but a reasonable estimate of their number could be made by means of fyke nets installed some distance downstream. Otherwise the weir was operated without difficulty throughout the entire period of smolt and adult migration.

The 1958 season was nearly a complete failure. Flood conditions delayed installation in the spring until the smolt migration was well under way. A log drive on the river caused much inconvenience and further delay, as well as causing some damage. A freshet on the first of October washed out a section of the fence, thereby permitting an unknown number of adults and possibly smolts to pass uncounted. About the only reliable data that was obtained was the count of hatchery-reared one-year-old smolts, which do not appear among the migrants until mid-May. As the weir was in operation within a day or two of the time these smolts had been observed in the past, and because the number taken was comparable to the numbers taken in previous years, it is believed that a reasonable estimate of their number was obtained.

The final tally of ascending adults in 1957 was 56, most of which passed through the weir in October and early November. Until the washout of October 1, the 1958 tally was 5 adults and 6 grilse, all unmarked. These 11 fish were very likely a substantial part of the total run for this year, for with the exception of three others taken by anglers, there were no authenticated reports of any having been seen below the weir as was the case in 1957. Undoubtedly there were a few but it is unlikely that the number was even close to that of the previous year. The validated rod catch in 1957 was 13, with unvalidated reports which would extend this figure to over 20.

For three successive years a seaward migration of a certain number of the hatchery fish planted the preceding fall has been observed. The number of these represents from 0.5 percent to

1.0 percent of the number planted. They are one year old from the time of hatching and are from 4.5 to 6.5 inches in length. They move directly into the estuary as do the older smolts and are able to adapt rapidly to marine conditions. Although constituting only a small fraction of the number planted, they make up about half the total number of seaward migrants surviving from any planting (based on 1956 and 1957 observations) and therefore must be considered an important segment of the population. It is interesting to note that these one-year-olds are from hatchery stock only.

The survival to be expected from them is low. No adults have ever been observed from Maine rivers which showed but a single winter's residence in fresh water on the scales. Furthermore, experiments conducted in Sweden on the survival of hatchery-reared smolts in the sea indicated practically no survival of fish less than 140 mm. (5.5 inches) in length at the time of release. This is the approximate mean size of the Sheepscot River one-year-old smolts.

During the summer of 1958 the weir was given a major overhaul, including the replacement of many of the supporting frames and modifications of the design to facilitate earlier installation in the spring.

Studies on the growth and survival of young salmon

The Sheepscot River is not an unfavorable environment for young salmon. Thermograph records have not indicated more than a few hours of temperatures close to the lethal level for salmon even in the hottest summers, while ordinarily summer temperatures seldom reach 80° F. The pH is only slightly acid and there is little silt or pollution. The food supply appears to be adequate and growth of young salmon in the river is excellent.

There are, however, large numbers of several kinds of predators, and some effort has been directed to discovering the nature and extent of predation.

The most abundant species of predatory fish is the common eel. Many investigators have claimed the eel to be a serious predator of young salmon. In the course of the predator studies made in the Sheepscot River over 100 eel stomachs were examined at different seasons of the year and none found to contain young salmon. Furthermore, at the time when the planted salmon would seem most susceptible to predation (immediately following their release) the eels have apparently begun their winter fast. Stomachs examined at that time are usually empty, and trapping by means of bait is much less successful.

The common pickerel (Esox lucius) is numerous in the river, especially in the pools. A few of these were taken by gill netting, and in one specimen, collected a few days after the salmon were planted, one salmon fingerling was found.

Kingfishers and great blue herons are numerous along the river, but no attempt has been made as yet to study their effect on salmon.

Mergansers, found to be definitely associated with salmon mortality in Canada, are now relatively scarce in the Sheepscot River area.

The sea lamprey is a possible predator. Although the adults cease feeding when they enter the river to spawn and die shortly after, the metamorphosed young, about six or seven inches long, have been observed on several occasions attacking alewives in the river. In the autumn of 1957 a great number of small lampreys were caught in the meshes of the downstream weir trap, all facing inwards, and appeared to be attempting to get at the fish inside.

The relationships among the salmon parr themselves need investigation. It is a fact that the larger salmon can and do prey upon the smaller ones and there is also the possibility of intraspecific competition for food and shelter. It is a common opinion that stocking hatchery salmonid parr in a river with a population of wild parr results in predation and/or competition to the disadvantage of the stocked fish. It is interesting to note with this in mind, that in 1954 the greatest number of marked salmon caught by anglers to date, in any river, was recorded for the Sheepscot. Since the Sheepscot was not extensively fished prior to this time, and it is known from subsequent observations that spawning runs occurred in 1952 and 1953, it is possible that marked fish

returned in those years also, though unobserved.

Until 1952, no runs of any consequence had been observed in the Sheepscot for many years and it is unlikely that during the period 1948-1952 wild parr were numerous in the river. Thus any fish planted during this period and due to return as adults in 1952-1954 enjoyed comparative freedom from the competition and predation of wild fish. The first returns from planted fish in the Sheepscot were expected in 1952, and by 1954 the offspring of these would be large enough to cause trouble. Fish planted from 1954 on have had to contend with a certain number of wild competitors, sometimes in great number.

An opportunity to look further into this question will be available this summer. Half of the 1958 planting was liberated above the dam at Coopers Mills, which has prevented spawning of Atlantic salmon in the river above it, with the result that in this area there are no wild salmon parr. The remainder of this planting were liberated below the dam as has always been done previously, and must contend with the wild parr present. If these areas are adequately sampled, the comparison may indicate whether there is any difference in survival.

Methods to circumvent the high mortality which seems to be the fate of planted salmon were investigated during the summer of 1958. It was thought that because (1) the smolt period and transition to a new environment might be critical, (2) the growth in salt water is rapid, and (3) smolts could be collected easily at migration time, a means of protecting smolts and taking advantage of their rapid growth to a size that would render them less susceptible to predation might enable a greater percentage of them to return to the river as adults.

As a preliminary experiment, two methods were tried. One of these consisted of closing off a section of a small tidal creek with slatted fences and sand bags to provide a natural estuarine habitat into which smolts could be released to feed on naturally occurring food organisms. The second method consisted of holding smolts in a large screened cage or live car, anchored in a protected cove near the laboratory in Boothbay Harbor and providing food in the form of immature sea herring, alewives and killifish.

The former method proved impracticable, at least on the small scale attempted. Scouring and undercutting by tidal currents, excessive tidal ranges, too much silt, the disinclination of forage fish to enter the area through the spaces of the enclosing fences, and fouling of these fences with debris made the project impossible without a prohibitive degree of maintenance and of construction expense.

The live-car method on the other hand showed some promise. The cages can be built relatively inexpensively and are not difficult to maintain. An adequate food supply can easily be collected during most of the season, and can be stored indefinitely in the cage along with the salmon. A means of improving the method by providing the live car with a system for trapping its own food supply from the dense schools of juvenile herring and alewives that are in the area from time to time throughout the summer will be tried this year.

Although the salmon placed in the car escaped by leaping out through the too-large meshes in the cover of the cage, which was designed primarily as a protection from birds, this fault can be readily corrected in the future. That salmon smolts will thrive and grow in captivity at a rate of nearly that under natural conditions was demonstrated by keeping several in an ordinary aquarium tank and providing them with all the small fish they would eat, amounting to about 5 small herring per salmon per day. One-year-old smolts 5 to 6 inches long when collected in May were 9 to 10 inches in length upon release in October.

This advanced rearing technique may be particularly useful in dealing with the smaller one-year-old smolts produced in the river, because as has been pointed out before, the natural survival to be expected of these is almost nil. If they can be held for a single summer, their size on release should be considerably greater than that of a naturally migrating two-year-old when it is first required to face a life in the ocean.

Two experiments for the purpose of studying survival of planted salmon were conducted in

connection with the 1957 and 1958 planting of salmon in the Sheepscot.

In 1957, the planted salmon were differently marked in two lots, each lot planted at widely separated localities on the river, between which stream population surveys of the previous summer showed no mixing of populations. The purpose of the experiment was to see if there was differential mortality in different parts of the river. The first smolts produced from this planting, the one-year-olds migrating in 1958, were about evenly divided between the two marked groups and the growth of these two groups also was almost identical. Thus it appears that, at least between the two localities tested (which are the customary planting sites) there is little difference in survival. More information will, of course, be available when the two-year-olds from this planting migrate this year.

The second experiment was to see whether the fish which migrate after a single winter in the river are recruited primarily from only the very largest planted fingerlings; or whether other factors are involved, so that any fish of sufficient size at planting may migrate the following spring, and that some of the very largest do not. This information might be of value in view of the fact that it is the intention of the hatchery people to produce parr for planting whose average size may be as large or larger than the largest individuals now produced.

The group for planting in the Sheepscot in 1958 was sorted into three size groups and differentially marked:

1087 very large parr over 4-1/4 inches long

1806 large parr 3-3/4 to 4-1/4 inches long

17,286 medium and small parr less than 3-3/4 inches long.

If most of the 1959 migration consists of the "very large" lot, and few or none of the others, and few or none of the "very large" lot are found remaining in the stream during the summer, it will be concluded that size at planting determines in a great degree whether the fish will remain another year or not.

If, on the other hand, the spring migration includes substantial numbers of both "large" lots, or of the "small to medium" lot, and fish of the "very large" group are found subsequently still in the river, size would seem less determinant.

It seems logical that inasmuch as only one or two hundred one-year-olds migrate to sea each year, if size is determinant, that the "very large" group alone would provide ample recruits for this migration, so that few, if any, of the other groups would be included. The basis for the size grades used in this experiment is the critical size of four inches suggested by Elson (1957) which the parr must attain before it can transform into a smolt for the next migration. The "very large" parr of the experiment are all in excess of this critical size. The "small to medium" parr are all below it. The intermediate size group are the "borderline" cases and the results of the experiment must be carefully interpreted if any substantial numbers of these "borderline" individuals are found among the first-year spring migrants.

Experiments on smoltification of salmon parr

If it becomes expedient to rear young salmon in salt water for direct release into the ocean, or for hatcheries to produce smolts, an understanding of the physiological and ecological processes involved in the metamorphosis from stenohaline parr to euryhaline smolts will be important. Much work has already been done in this field and among the important discoveries is that artificial "smolts" which can withstand high concentrations of salt water have been produced in salmonids by feeding them diets rich in iodine.

It seemed possible that dissolved iodine salts in the water in which parr of large enough size were held might have a similar effect. An experiment was carried out in the summer of 1958 to test this theory.

A group of 5- to 6-inch salmon parr from the Sheepscot River were subjected to a treatment of dissolved sodium iodide in the water, but after 30 days of such treatment showed no more ability to withstand salt water than did the controls. In

48 hours both groups had succumbed completely to a salinity brought gradually up to about 32°/°°.

Estuarine ecological studies

A part of this work has been devoted to the study of the seasonal availability of organisms suitable for use as salmon food.

Much of the effort in the field of estuarine ecology has been in the study of the effects of river discharge on the hydrography of the upper estuary and on the biotic communities living therein. Most of the field work for these studies was completed in 1957, but additional data was collected in 1958 which shows a marked difference between the two years. This difference is undoubtedly due to the fact that while 1957 was one of the driest summers of recent years, 1958 was unusually wet.

The laboratory work and analysis of the data for this ecological study is currently being carried on and the results will be eventually incorporated into a manuscript for publication.

Future prospects

The objectives of this investigation will continue to be the understanding and reduction of mortalities of young salmon, with perhaps further accent on the smolt stage and salt-water growth and survival. Many of the possible lines of research will depend on funds and personnel available. The weir will be kept in operation as long as possible, but if this project is to be continued, the possibility of rebuilding or replacing it must be given some study.

In recent months there has been an expressed desire on the part of certain sportsmen's groups to take a more active part in salmon restoration work. These groups have volunteered to assist both Federal and State agencies in any practicable way and have even requested specific assignments. One group has made several proposals of projects in which they felt they could provide assistance.

It seems desirable that this feeling of cooperation should be encouraged and that there should be several activities in which non-professional, interested people could be given a share. Some of these might be: (1) assistance in weir maintenance; (2) assistance in stream surveys with electric shocking gear; (3) assistance in the collection of data from fish caught by anglers; (4) assistance in distributing fish at the time of planting.

On November 21 a conference attended by biologists of the Maine Salmon Commission, Department of Inland Fisheries and Game, Sea and Shore Fisheries and Salmon Investigations of the Fish and Wildlife Service was called to meet with representatives from two local sportsmen's groups to discuss these matters, and at that time some of the above listed projects were suggested.

One of the proposals made by several of the sportsmen seems to have some merit as a project provided sufficient funds are available and provided a workable arrangement could be made with the sportsmen's group for their cooperation. This scheme would consist of purchasing adult salmon from commercial fishermen in the Small Point area and using these as brood stock. A modification of this proposal might perhaps be more satisfactory, namely to transfer the salmon to the river to add to the natural spawning stock. The weir, of course, would prevent their return to the sea. This project would be set up as an experiment with these objectives:

(1) To see whether adult salmon could be expediently collected, transported and adjusted to fresh water.

(2) To see if once in the river salmon of alien stocks will ascend to the spawning grounds in a strange river.

Preliminary trials of such a project could be carried out in 1959 with little expense.

The salmon which are taken commercially at Small Point seem to be an ideal source of brood stock, since in that way nothing is removed from the spawning stock of other rivers that would not be lost to the market anyway; furthermore, evidence from earlier tagging studies shows that many of these commercially taken salmon are not

native to Maine rivers, and even if allowed to go free, they would contribute nothing to these rivers.

Further experimentation on the holding and growing of salmon smolts in live cars is definitely planned for the 1959 season.

Stream surveys by electroshocking to supplement the routine survey made by the Salmon Commission, and to provide answers to specific problems, some of which have already been outlined are also planned.

Further experimentation in the field of smolt physiology should also be carried out, but experiments of more than an elementary nature will not be attempted with the present facilities and personnel.

The manuscript for a paper titled "Introductory Report on the General Ecology of the Sheepscot River estuary, Maine" has been completed and submitted to Washington for approval.

Literature cited

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California-Nevada Sport Fishery Investigations
Reno, Nevada
Reed S. Nielson, Chief

These investigations are designed to evaluate the extensive practice of stocking hatchery-reared, catchable-size rainbow trout in streams, to compare the survival of trout reared on meat and dry diets at various hatcheries, to determine the effects of seasonal conditions on trout and on their stream habitat, and to develop new techniques for the assessment of alpine stream and lake productivity. A mile-long, four-section, experimental stream area is used for studies of stream carrying capacity and the trout vitality-viability complex. The stream is subject to complete screen control and, by draining, to population counts. Lake productivity studies are conducted on alpine waters of the eastern Sierra Nevada range.

During the period of this report the survival studies (stream, pond, and starvation) have been mainly directed toward a better understanding of the importance of hatchery diet to the subsequent survival of stream-planted, catchable-size rainbow trout. Attention has also been given to the nature and extent of conversion to natural feeding of such trout when placed in mountain streams.

The sum of our data to date indicates that a hatchery diet of meat and fish products generally results in trout of better survival qualities than those raised on dry food, but also suggests that a dry food formulated to give equivalent nutritional quality would produce trout of equal or almost equal vitality. Stream tests of several lots of commercial-pellet-fed trout were characterized by heavy mortalities and extreme weight losses, suggesting nutritional deficiency damage that was not rectified by the shift to natural foods.

An additional two-week survival experiment demonstrated that early post-planting mortality of initially healthy and properly handled trout delivered at Convict Creek was not appreciably affected by hauling time, density of fish in the load hauled, or by the use or non-use of drugs and anesthetics carefully administered.

Work on lake productivity included further limnological and biological observations on an experimental lake at 11,000 feet, establishment of five limnological stations on Crowley Lake,

collection and analysis of periodic periphyton samples from Convict Lake, collection and analysis of water to represent temperature-controlled distribution of organic matter in several alpine lakes, testing and calibration of photometer components (submersible and non-submersible), development and testing of a wet oxidation technique to characterize lake organic matter, and development and testing of a microfiltration system for obtaining oxidation samples of plankton.

SURVIVAL AND VITALITY OF HATCHERY TROUT

1957-58

Experiments to compare the survival of catchable-size rainbow trout from four California Department of Fish and Game hatcheries (Moccasin Creek, Fish Springs, San Joaquin, and Hot Creek) under controlled natural stream (running water) and pond (quiet water) conditions were terminated May 1 - 4, 1958. Each hatchery furnished 150 pounds of trout except that two such lots were obtained from Moccasin Creek Hatchery to compare trout reared exclusively on pellets with those produced on a standard meat diet. Tests with the Moccasin Creek trout were initiated May 24, 1957, while those from the other hatcheries were begun July 29, 1957 to duplicate last year's (1956-57) experiments. The four experimental stream sections were stocked at a density of 200 pounds per acre and trout from each hatchery source were equally represented by weight. Fifty trout from each of the Maccasin Creek Hatchery groups and 30 from each of the other hatchery lots were stocked in a natural holding pond, and 50 from each lot were held in fine-mesh enclosed starvation troughs.

Moccasin Creek Trout

At the end of 64 days (July 18) of stream residence the survival of pellet-fed trout in one stream section was 93.6 percent as compared with 79.8 percent for those reared on the standard meat diet. Poaching on the other stream section in which Moccasin Creek trout were stocked necessitated the recovery and counting of trout from that section on August 21, 1957. Thus no true survival data were obtained from these trout for their initial 98 days in the stream. However, the experiment was continued with the survivors and their survival was checked on November 21 (103 days). At this point the survival was 88.9 percent for pellet-fed trout and 88.4 percent for the meat-fed group. The 162-day overwinter survival (November 21, 1957 - May 2, 1958) was 52.8 percent for meat-fed trout as compared with 33.0 percent for those fed on pellets.

The pond test continued for 345 days (May 24, 1957 to May 4, 1958). At the conclusion of this experiment the survival of the pellet-fed group was 24.0 percent and that of the meat-fed group was 34.0 percent. This is the first instance in which the survival of trout in the pond was poorer than that in the stream.

In a 232-day starvation test (May 24, 1957 to January 10, 1958) the percentage survival for both diet groups was 16.0.

On the basis of the results of these experiments, it is concluded that there was no significant difference in the hardiness of the two diet groups. It follows that the effective nutritional quality of the pellet diet was equal or nearly to that of the standard meat diet used.

San Joaquin, Fish Springs, and Hot Creek trout

Trout from these hatcheries were initially stocked in three experimental stream sections at 200 pounds per acre on July 29, 1957. Snow and ice conditions made it impossible to check survival on November 1, 1957 as scheduled and this operation was accomplished November 20. The following summary compares the percentage stream survival of trout reared at these hatcheries in two successive years:

Hatchery	July 29-Nov. 1, 1956 94 days	July 29-Nov 20, 1957 115 days
San Joaquin	97.1	70.3
Fish Springs	91.7	90.8
Hot Creek	96.3	94.8

On the basis of these tests and in consideration of the longer test period this year, it is obvious

that the Fish Springs and Hot Creek trout were of equal quality for two years of production. However, this is not the case with the San Joaquin trout, and the only difference between the lots of the two years was found to be in the diets fed. In 1956 the trout from this hatchery were reared exclusively on the standard meat diet. In the 1957 lot the standard meat diet was initially fed and then shifted to an almost exclusive pellet diet (same type of pellet fed at Moccasin Creek Hatchery) for the last six months of hatchery residence.

The survivors at the November 20 check point were restocked in two experimental stream sections for over-winter survival. This phase of the experiment was terminated May 3, 1958 (163 days) with the following results in percentage survival, compared with the results obtained the previous year:

Hatchery	Nov. 1, 1956 - April 30, 1957 (181 days)	Nov. 21, 1957 - May 3, 1958 (163 days)
San Joaquin	69.3	11.0
Fish Springs	21.9	22.4
Hot Creek	23.1	41.3

The most striking situation here is the very poor survival of the San Joaquin trout as compared with their excellent performance in 1956-57. The performance of Fish Springs trout was the same for both years, and Hot Creek trout showed decided improvement in survival this year. If poor survival of San Joaquin and Fish Springs trout is related to nutritional deficiency in hatchery diets, then it may be concluded that these trout failed to recover from nutritional damage despite the change to natural foods in the stream.

Thirty trout from each hatchery group were stocked in the holding pond July 29, 1957 and retained there until the experiment was terminated May 4, 1958 - 280 days. In the following summary, the percentage survival of the various groups can be compared with their performance in the previous year (July 28, 1956 to April 30, 1957 - 276 days).

Hatchery	1956-57	1957-58
San Joaquin	100.0	16.7
Fish Springs	63.3	63.3
Hot Creek	53.3	63.3

The 1857-58 results were of the same order as those obtained in the stream experiments. San Joaquin trout performed poorly. Performance of Fish Springs trout was the same for the two years, and there was some improvement in the survival of Hot Creek trout.

In a 166-day starvation test (July 29, 1957 to January 10, 1958), the percentage survivals for the three groups were: San Joaquin 26.0, Fish Springs 16.0, and Hot Creek 26.0.

Hauling and handling of hatchery trout

In addition to the long-term survival tests, a two-week experiment (May 12 - 27) was conducted to test early post-planting mortality as affected by the weight of trout carried in tank trucks, length of haul and hauling time, and the amount of handling sustained by the trout prior to actual release in the stream. Two lots of catchable-size rainbow trout (light and heavy loads) each from Fish Springs and Moccasin Creek Hatcheries were used. Results indicated that:

1. Trout transported in light loads (150 pounds) such as are delivered to Convict Creek for experimental purposes have no measurable advantage, in terms of survival, over those carried in heavy loads (300-350 pounds) hauled in routine stocking operations in 150-gallon tanks mounted in pickup trucks.

2. Handling, hauling (drugged - sodium amytal - and undrugged), anesthetization (chlorobutanol) for marking, measuring, and weighing, when carefully conducted, have no appreciable effect on early mortality of initially healthy and robust trout.

3. A long haul (12-1/2 hours), light or heavy load, is no more likely to result in so-called delayed planting mortality than a short haul (1-3/4 hours) at the same load level, again assuming that the trout were in good physical condition and were properly handled.

1958-59

New long-term survival experiments with catchable-size rainbow trout (origins and diet data indicated below) were initiated on August 1, 1958 and will be terminated in early May 1959. These tests have the same design as those conducted in 1957-58; that is, the stream sections were stocked at 200 pounds per acre, the starvation test employed 50 trout from each hatchery source, and the pond was stocked with 30 trout from each hatchery.

Hatchery	Egg source	Diet
Fish Springs	Hot Creek Fall-Spawning	Fish 6 days, Meat 1 day
Mojave	Hot Creek Fall-Spawning	Pellets
Shasta	Shasta Fall-Spawning	Pellets
Black Rock	Mt. Whitney Spring-Spawning	Fish 5 days, Meat 1 day, Blank 1 day

Each hatchery furnished 150 pounds of trout, and all arrived in good condition with no losses in hauls ranging from 2-1/2 to 15-1/2 hours. Average total lengths of the trout selected for experimental use ranged from 20.0 to 21.2 centimeters (7.9 - 8.3 inches).

Shasta trout were below average in body condition ("K" = 0.82 as compared with 0.96 -- 1.02 for the other three groups). Mojave and particularly Black Rock trout did not handle well, and in combination these two groups sustained a two percent loss during the 6-day preliminary holding and marking period. That these early indications of low vitality are important to survival is apparent in the results of the routine November population checks as follows:

Hatchery	Percent Survival, Aug. 1 - Nov. 7 (99 days)		
	Stream Test	Starvation Test	Pond Test
Fish Springs	94.0	88	97
Black Rock	89.3	0	100
Mojave	79.0	18	93
Shasta	58.8	0	90

In the stream test, the pellet-fed Shasta trout lost weight rapidly and suffered the heaviest mortality; it is clear at this point that their overwinter survival will be very low. In the starvation test, all Shasta trout were dead by September 11 (42 days) and all Black Rock trout were dead by November 2 (94 days). The starving Mojave trout were reduced by steady losses to 18 percent of original number by November 7, and to 14 percent by December 31 (153 days), at which time 82 percent of the Fish Springs trout still survived. As in previous tests, the trout raised on meat and fish at Fish Springs Hatchery showed excellent vitality through the fall months and it appears likely that their overwinter survival will be determined by the severity of stream conditions rather than by a change in their own lasting qualities. Experience tells us that the early decline of other groups under starvation conditions will mean their steady depreciation in the stream regardless of winter severity.

Losses in the holding pond, where exertion is at a minimum and some natural food is available, were small in all groups as usual.

Following the November population check the stream test was reduced in size to facilitate winter care of the area. A 10-day experiment to compare feeding propensities among several groups of wild and hatchery-reared trout was conducted in one of the remaining open stream sections. Analysis of the stomach contents of these and other trout is now in progress as part of the continuing study of the food-survival relationship in stream-dwelling trout.

PRODUCTIVITY OF HIGH SIERRA LAKES

Field work in the 1957 season included observations and collections to complete a 6-year history of brook trout and their organic environment in Bunny Lake, a high-altitude cirque of extremely low water fertility (report published). Other work established five observation stations

and provided preliminary limnological information on Crowley Lake, an eastern Sierra reservoir which is also a very productive trout water.

In the 1958 field season attention was given to equipment and techniques pertaining to lake productivity assay, particularly concerning the phytoplankton component.

A lake photometer (standard oceanographic type) was found unsuitable for measuring solar radiation characteristics in high-altitude lakes because of the great light intensity there. This device was modified for such use by redesigning the shunt circuit and by adding neutral density filters in the form of screens and opal glasses. Calibration of these elements, together with preliminary field tests, indicated that reduction of incident light to about one percent is necessary in order to operate the photometer with present circuit design. Accurate calibration of the sea-cell (submersible) components with existing facilities proved too difficult; light readings on Convict Lake, therefore, gave only relative depth-illumination curves. Attempts to characterize the radiation in lakes of the upper basin (above 9,000 feet) showed that the photometer is rather cumbersome to operate from a rubber life raft, and that ideal wind and sky conditions are requisite, especially in clear or shallow water. Further modifications on the photometer seem necessary in order to adapt it to use on alpine lakes.

A wet oxidation technique was developed to characterize better the lake organic matter (both particulate and dissolved). Early limnologists used permanganate oxidation to determine the amount of organic matter present in a sample of water. This was described as being 40 percent effective in oxidizing the organic carbon present. The presently developed technique employs a strong acid-dichromate mixture to oxidize quantitatively dried organic matter such as phytoplankton, seston, or evaporation residue. It has the following features:

Sensitivity and effectiveness: Organic matter is quantitatively oxidized to 95 - 100 percent effectiveness over a wide range of sample sizes. The micro-to-macro scale is accomplished by merely adjusting the normality of the dichromate solution used while keeping the acid level constant (ca. 75% conc. H_2SO_4). Fats, purified proteins, and chemically pure amino acids were oxidized to values approaching 100 percent of theoretical oxygen requirements, with a precision of $\pm$ 5 percent. The analysis has been modified at the Station laboratory for greater sensitivity in order to detect small differences in the organic content of these organically poor lakes.

Applicability: This technique is designed either to supplement or replace the more common standing crop measurements of plankton (numerical, gravimetric, volumetric), and is further applicable to organic matter in any of its forms as found in a lacustrine environment. (a) As an independent measurement: the quantity of organic matter in a given volume (or on a given surface area) is estimated in grams by multiplying the volume of 1N dichromate required in oxidation by the factor .0069. Also, the percentage organic matter in any admixture of mineral and organic substances of known weight can be similarly approximated. (b) As a supplementary technique: The oxygen requirement of any given weight of organic matter is directly related to the energy content of that sample, such as caloric value. In other words, the amount of oxygen required to oxidize a given organic sample represents the degree of reduction of carbon present (therefore the energy stored). Thus, the dichromate oxygen demand of a given weight of plankton is a standing-crop measurement of the amount of energy present.

Oxidation values of the samples thus far analyzed indicate only slight differences in organic content among the four lakes studied. These values range from one to three milligrams per liter of oxygen consumed (multiplied by the tentative factor, 1.5, gives the amount of organic matter in milligrams per liter). The analyses also show that approximately four-fifths of the total organic matter occurs in a dissolved state. The most striking differences in organic content occurred in Cloverleaf Lake which has four partially isolated, shallow basins, each of a slightly different temperature. Therein, the total organic content varied directly with temperature.

Since the basic organic components of an organism (fat, protein, carbohydrate) each represent a different degree of carbon reduction, each

has a different oxygen demand. By combining the results of dichromate oxidation and of organic nitrogen analysis, the amounts of these basic components in any sample can be determined.

Microfiltration via millipore filter technique has demonstrated its usefulness in Convict Creek Basin water in separating the particulate matter from dissolved substances, and appears superior to other techniques in numerical estimation of plankters, especially nannoplankters. It is intended to combine microfiltration with quantitative dichromate oxidation to survey the standing crops of plankton in these lakes. This sampling will be done in conjunction with temperature and photometric light measurements. The results are expected to provide a further measure of lake productivity and to provide a comparison for productivities established by the periphyton technique.

Work was continued on identification, distribution, and methods of mounting and preserving periphyton organisms.

Rocky Mountain Sport Fishery Investigations
Logan, Utah
Oliver B. Cope, Chief

This Investigation has been concerned with three principal programs during the period covered by this report. The greatest effort has gone into the continuing studies in Yellowstone National Park, and has been directed toward population problems on Yellowstone Lake and its tributaries, on the Madison River system, and on a few smaller waters in the park. The second program has been carried on in Rocky Mountain National Park, where much interest has been generated on the restoration of the green-back trout. The third segment of the work has been concerned with pesticides, including field and laboratory investigation of the effects on fish of a DDT spray program in the Yellowstone River drainage and including work on the establishment of a new pesticide laboratory for studies on economic poisons, fish, fish foods, and water.

YELLOWSTONE LAKE AND TRIBUTARIES

Limnological studies

Studies on the limnology of Yellowstone Lake was one of the primary efforts in this drainage in 1957 and 1958. This work, a continuation of observations and measurements made in earlier years, resulted in considerable data which helps explain the behavior of cutthroat in Yellowstone Lake.

Mapping of lake:--Mapping of the lake was completed in the field, and five-fathom contours were located on a chart. The lake has an area of 135.77 square miles, a maximum depth of 320 feet, a mean depth of 139 feet, and a total capacity of 12,095,264 acre feet of water. Most of the bottom food organisms are located at depths of less than 60 feet. The amount of lake bottom, 60 feet or less in depth, occupies 25.6 percent of the lake bottom.

Temperature and currents:-- Water temperature measurement was continued to add to data compiled since 1940. Surface temperatures above 66° F. have never been recorded. The lake completely freezes over each winter and the ice leaves from early May to early June. The lake begins to stratify in late June and early July. During late July complete stratification exists. The thermocline begins to lower in early September, and a slight trace is visible in mid-October.

The horizontal variation in temperature stratification is directly related to currents developed by wind. Drift bottles have clearly demonstrated the predominant surface water movement from the west to the eastern section of the lake. These water movements cause a shallow epilimnion and an indistinct thermocline in West Thumb and deep epilimnion and distinct thermocline in the east section of the lake. Intermediate thermal conditions occur in other sections of the lake. Variations year to year fluctuate within narrow limits.

Water chemistry:--Oxygen concentrations were usually above 90 percent saturation at depths down to 200 feet. Bicarbonate alkalinities ranged from 22 to 41 ppm., with the lowest readings in the Southeast Arm and the highest readings in West Thumb. Free carbon dioxide was present in all samples and varied from 1.5 to 13.3 ppm. Other abundant ions are silicon, calcium, and sodium. Yellowstone Lake can be classified as morphometrically oligotrophic, a term which does not necessarily indicate low productivity.

Plankton:--Daphnia, Bosmina, Diaptomus, Conochilus, and Vorticella are all abundant in Yellowstone Lake and make up over 99 percent of the zooplankton. All are abundant down to a 75-foot depth, but are rare below 100 feet. The common phytoplankton genera are Asterionella, Melosira, Stephanodiscus, and Anabaena. Asterionella is most abundant during June, while Anabaena is abundant in August. The Southeast Arm, which had the lowest alkalinities, has the lowest plankton populations. West Thumb appears to be the most productive area.

Bottom organisms:-- Gammarus lacustris, Tendipes (Tendipes), Tendipes (Limnochironomus), Procladius, and Prodiamesa are the most abundant organisms, although Ephemerella spp. and several limnophilid caddis flies are common in water less than 25 feet in depth. The greatest production is in the upper 50 feet of water. Aquatic plant beds, particularly Ceratophyllum demersum and Potamogeton pusillus, are especially productive of bottom organisms.

Food of cutthroat trout:-- The most abundant organisms in the 400 cutthroat stomachs examined were Daphnia, Diaptomus, Tendipedidae pupae and adults, and Gammarus lacustris. Gammarus was most common and was present in the stomachs during all months of collection. Four stomachs contained trout fingerlings, and several stomachs contained trout eggs.

Distribution of trout:--Reliable data on the horizontal distribution of the trout have not yet been collected, although gill net density figures show that the northern section has the largest population. Gill net catches have shown that the water less than 40 feet in depth contains over 75 percent of the trout population. These data agree closely with that from food studies on trout stomachs, and from our knowledge of the food production in the lake.

Primary production:--Efforts were made to measure primary production by the "light and dark bottle" technique and by suspending glass slides. The bottle technique gave conflicting results, and the glass slides have not yet been studied. The "effective depth" of Yellowstone Lake where most of the organic production occurs can be tentatively stated to be in the upper 75 feet of water.

Anglers' catch

The catch of trout in Yellowstone Lake and River during 1958 increased 27 percent over any former year measured (table 1) (fig. 1). This catch was coincident with a 9.7 percent decrease in numbers of visitors to Yellowstone National Park from 1957 to 1958. The hours of fishing effort increased from 568,152 in 1956, the biggest previous year, to 739,463 in 1958, or 23 percent. These data point to a significant increase in fishing interest in Yellowstone National Park and indicate the necessity for following the fishery in the future.

The spawning runs

Spawners counted in six streams in 1958 showed that they are continuing to maintain their numbers at safe levels, despite increasing fishing pressure. Table 2 presents the history of the counts from 1950 to 1958, and shows that the decline of a few years ago has leveled off and perhaps moved upward. This encouraging trend has occurred during a period of heavy increase in fishing pressure. Another feature of the spawning runs of 1958 was the large sizes of the spawners. Average lengths of fish were significantly greater than those of average years. Scale studies to determine changes in age composition of these populations will be initiated soon.

Growth rate

Studies were continued on growth rates of cutthroat from Yellowstone Lake to determine accurately the body-scale relationship and to gather additional information on age composition of the catch and of the spawning runs.

Table 1. Number of tourists visiting Yellowstone National Park, and hours of fishing and number of fish caught on Yellowstone Lake and River from 1950 to 1958.

Year	Number of tourists	Hours of fishing	Number of fish caught
1950	1,109,926	292,989[1/]	200,015[1/]
1951	1,163,957	436,704	227,984
1952	1,350,295	494,426	265,880
1953	1,326,858	413,348	223,805
1954	1,328,893	408,138	251,845
1955	1,368,515	520,898	349,757
1956	1,457,782	568,152	312,128
1957	1,595,875	513,032	343,116
1958	1,442,428	739,463	481,093

[1/] Does not include Yellowstone River fishing pressure or catch.

Table 2. Numbers of spawners entering six streams from Yellowstone Lake, 1950-1958.

Year	Pelican Creek	Clear Creek	Chipmunk Creek	Grouse Creek	Cub Creek	Arnica Creek	Total
1950	15,076	10,459	12,255	9,635	2,179	3,881	53,485
1951	9,423	16,879	12,585	12,188	2,676	4,755	58,506
1952	7,953	10,269	9,989	6,949	2,056	3,523	40,739
1953	12,418	10,323	7,836	7,441	3,848	4,524	46,390
1954	10,340	3,161	5,371	3,756	3,446	6,200	32,274
1955	12,400	7,929	3,880	--	2,558	4,700	--
1956	7,000[1/]	--	--	--	--	3,912	--
1957	17,500	8,398	9,605	--	2,976	3,812	--
1958	7,259[1/]	9,355	8,922	13,462	1,747	4,041	44,786

[1/] Count known to be low.

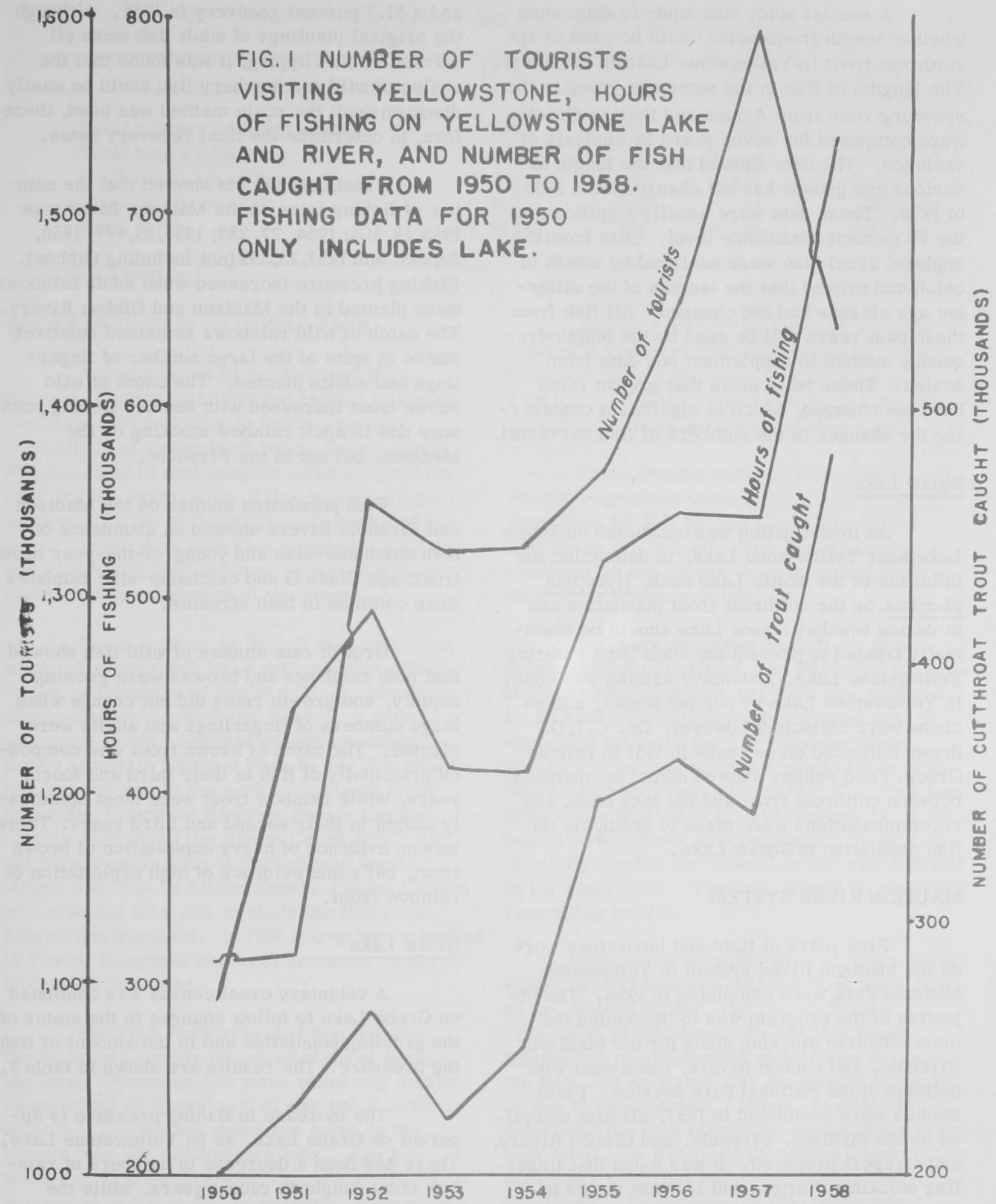


FIG. 1 NUMBER OF TOURISTS VISITING YELLOWSTONE, HOURS OF FISHING ON YELLOWSTONE LAKE AND RIVER, AND NUMBER OF FISH CAUGHT FROM 1950 TO 1958. FISHING DATA FOR 1950 ONLY INCLUDES LAKE.

A special study was made to determine whether length frequencies could be used to age cutthroat trout in Yellowstone Lake in the future. The lengths of fish in the same age group in the spawning runs from Arnica and Pelican Creeks were compared for seven years by analysis of variance. The data showed that the length of various age groups has not changed from 1950 to 1956. These data were usually significant to the 99 percent confidence level. Data from the anglers' creel also were analyzed by month of catch and proved that the lengths of the different age classes had not changed. All fish from these past years will be aged by the length-frequency method to supplement our data from scales. These facts mean that growth rates have not changed, which is significant considering the changes in the numbers of fish harvested.

Squaw Lake

An investigation was conducted on Squaw Lake, near Yellowstone Lake, to determine the influence of the exotic Lake chub, Hybopsis plumbea, on the cutthroat trout population and to decide whether Squaw Lake should be chemically treated to prevent the chub from entering Yellowstone Lake. Extensive seining was done in Yellowstone Lake by our personnel, and no chubs were collected; however, Dr. C.J.D. Brown collected these chubs in 1951 in Pelican Creek. Food studies showed direct competition between cutthroat trout and the lake chub, and recommendations were made to eradicate the fish population in Squaw Lake.

MADISON RIVER SYSTEM

Five years of field and laboratory work on the Madison River system in Yellowstone National Park were completed in 1958. The objective of the program was to determine the most effective stocking plans for the Madison, Firehole, and Gibbon Rivers, consistant with policies of the National Park Service. Field studies were completed in 1957, all data compiled on the Madison, Firehole, and Gibbon Rivers, and a report prepared. It was found that fingerling stocking of brown and rainbow trouts in these streams did not contribute to the fishery. Adult stocking of rainbow trout in the Madison River showed a 33.2 percent recovery in 1954 and a 51.7 percent recovery in 1955. Although the original plantings of adult fish were all marked by fin clipping, it was found that the scales of wild and hatchery fish could be easily distinguished; the scale method was used, therefore, to determine the final recovery rates.

Creel census data showed that the number of fishing trips on the Madison River was: 1953, 19, 504; 1954, 27, 233; 1955, 23,477; 1956, 24,293; and 1957, 25,599 (not including Gibbon). Fishing pressure increased when adult rainbows were planted in the Madison and Gibbon Rivers. The catch of wild rainbows remained relatively stable in spite of the large number of fingerlings and adults planted. The catch of wild brown trout increased with heavier fishing pressure due to adult rainbow stocking on the Madison, but not in the Firehole.

Fish population studies on the Madison and Firehole Rivers showed an abundance of both catchable-size and young-of-the-year brown trout; age Class O and catchable-size rainbows were common in both streams.

Growth rate studies of wild fish showed that both rainbows and browns were growing rapidly, and growth rates did not change when large numbers of fingerlings and adults were planted. The catch of brown trout was composed principally of fish in their third and fourth years, while rainbow trout were most commonly caught in their second and third years. There was no evidence of heavy exploitation of brown trout, but some evidence of high exploitation of rainbow trout.

Grebe Lake

A voluntary creel census was continued on Grebe Lake to follow changes in the status of the grayling population and in the amount of fishing pressure. The results are shown in table 3.

The increase in fishing pressure is apparent on Grebe Lake, as on Yellowstone Lake. There has been a decrease in numbers of rainbow trout caught in recent years, while the grayling catch has increased.

Table 3:--Creel census data from Grebe Lake, 1953-1958.

	1953	1954	1957	1958
Total number of fishing trips	363	411	565	1,212
Total hours of fishing	1, 256	1, 317	1, 644	2,098
Total catch, grayling	492	1, 083	1, 371	1,182
Total catch, rainbow	362	350	255	283
Total catch	1, 237 1/	1, 433	1, 626	1,465

1/ 383 unidentified

ROCKY MOUNTAIN NATIONAL PARK

At the request of the National Park Service, a study was initiated in 1957 in Rocky Mountain National Park to locate existing populations of green-back trout, Salmo clarki stomias, and to find suitable waters within the park for the restoration of this native subspecies. An investigation of the nature and extent of the fishery in the park was also requested. By the end of the 1958 field season, progress had been made on several phases of the study.

The discovery in 1955 of fish suspected to be the green-back trout was made in Albion Creek, a tributary of Boulder Creek, which drains into the South Platte River. A survey was made in 1957 and 1958 of waters within Rocky Mountain National Park to see if similar fish existed within the park. Cutthroat from upper Big Thompson River in Forest Canyon closely resembled the Albion Creek fish. As the population in Forest Canyon was much larger than that in Albion Creek, Forest Canyon was selected as a site to study the fish in its natural environment. In 1958 a trap was installed in Forest Canyon to obtain information on the biology of this trout.

The 1958 study revealed that the cutthroat in this stream do not migrate at spawning time. Spawning fish were relatively small; mean length of spawning fish was 190.8 millimeters. The largest specimen taken in the stream was 236 millimeters long. Age frequency of 41 spawners was: Age Class III, 71 percent; Age Class IV, 29 percent. Males were predominant in the spawning population. Spawning commenced about July 1, and was completed by July 15. First fry were observed on August 26.

Summer diet of the Forest Canyon fish consisted predominantly of terrestrial organisms, with adult Hymenoptera as the major food item. Aquatic organisms were taken in greatest numbers during the spring runoff.

Mean lengths at capture in millimeters for each age class were: Age Class I, 93.12; Age Class II, 130.13; Age Class III, 173.59; Age Class IV, 205.40. The Forest Canyon cutthroat do not appear to form scales during their first summer of life.

To study the green-back trout in both lake and stream habitats, the Fay Lake drainage in Rocky Mountain National Park was selected as an area for restocking this subspecies. The upper 2.2 miles of the Fay Lake drainage contains three lakes which are similar to many other montane lakes in the park. The area was chemically treated in 1958 to remove the resident populations. Potassium permanganate was successfully used in the stream to restrict fish kill to the study area. The area should be ready for restocking in 1959.

Specimens of cutthroat trout from waters within Rocky Mountain National Park were sent to Dr. Robert Miller, Curator of Fishes, University of Michigan. Dr. Miller found interesting morphological differences between the fish, but he stated that the differences may be due to environment. Hence, in order to determine the true taxonomic status of the green-back trout, the fish must be reared concurrently with other cutthroat subspecies under controlled conditions. To accomplish this important phase of the project, and to determine if green-back trout can be reared in the hatchery, 26 Albion Creek fish were transported in 1957

to the U.S. Fishery Station at Leadville, Colorado. Some of these died during the winter, and the survivors did not spawn in 1958, although they did grow well. In 1958, 50 adult cutthroat from Forest Canyon were taken to Leadville. If they spawn in 1959, eggs of this variety will be hatched and reared concurrently with young of the Colorado River cutthroat, Salmo clarki pleuriticus. If anatomical differences are found in the resulting fish, the status of the greenback trout may be clarified.

During the general survey of park waters to locate possible populations of green-back trout and to locate suitable areas for stocking this subspecies, some information was obtained on the fish populations now existing in these waters. Six streams and seven lakes were examined in 1957; in 1958 six streams and 17 lakes were surveyed. All of the lakes examined were relatively small (1.0 to 27.0 acres) and slightly acidic (pH from 5.5 to 6.8). Methyl orange alkalinity varied from 4.5 to 13.0 ppm. Little or no spawning gravel is available in most of the lakes. Only brook trout appeared to have much spawning success in the lakes, with one exception in the Fay Lake drainage where cutthroat were spawning successfully. Most of the lakes require periodic planting to maintain populations. Brook trout are reproducing well in streams containing this species. They are usually the most abundant species, when present. All of the streams surveyed had relatively small numbers of aquatic invertebrates. The summer diet of trout in most of the lakes and streams was predominantly terrestrial organisms.

In 1958 a creel census was conducted on waters within the park east of the Continental Divide to determine the amount of fishing pressure, species composition, mean lengths, and size of the harvest. Four streams received most of the fishing pressure: Big Thompson River, Fall River, Glacier Creek, and the North St. Vrain River. Fishing pressure and harvest estimates for these streams are given in table 4, where mean length in inches is in parentheses

Fishing pressure on lakes was less than on the streams, and catch per hour was higher than for the streams. Most of the catch from the streams was composed of wild brook trout. Planted rainbow trout were the next most important species. Approximately 21 percent of the harvest from the four streams listed above consisted of rainbow trout planted as fingerlings in 1957.

PESTICIDE STUDIES

Spruce budworm

The Forest Service and National Park Service sponsored a DDT spray program in and around Yellowstone National Park in 1957 for the purpose of controlling spruce budworm which was infesting Douglas fir. This program featured spraying from airplanes in 26 areas involving 72,000 acreas of forested terrain. Several streams, small and large, flow through or near the sprayed areas, and important fish populations inhabited waters in the spray areas and in downstream stretches. Since dead fish were found in some of these streams after a 1955 spraying, and since 1956 studies in Montana had demonstrated that similar spray programs caused serious reductions in aquatic invertebrate populations, it was decided that observations should be made in connection with the 1957 spray program to determine the extent of any damage to fish or fish food. Collections and observations were made before the application of DDT in mid-July, periodically through the summer of 1957, and throughout the summer of 1958.

Collections of stream-bottom invertebrates and those drifting in the stream showed that severe reductions took place after the spray had been deposited, and it is concluded that the DDT spray was responsible. Repopulation of affected streams was measured in late-season sampling, and full recovery was found in some streams through sampling in 1958.

Chemical analysis in exposed waters showed DDT to be present in several samples from streams, and the highest concentration was found in a lake sample. One water sample taken 55 miles below the spray area contained a trace of DDT. Chemical analysis of three aquatic vegetation samples from streams showed DDT to be present in all cases.

Thirty-three fish were macerated, blended, and analyzed chemically; all contained DDT. Whitefish and rainbow and brown trout taken from

Table 4.--Effort and catch estimates for Rocky Mountain National Park streams, 1958.

Area	Total fisherman hours	Harvest				
		Brook	Rainbow	Brown	Cutthroat	Total
Big Thompson River	8,924	4,567 (7.04)	952 (8.01)	287 (9.45)	129 (6.66)	5,935
Fall River	8,453	3,926 (6.98)	1,152 (6.74)	200 (7.78)	26 (6.00)	5,304
Glacier Creek	8,039	2,525 (6.63)	1,341 (7.58)	66 (8.50)	395 (10.20) 1/	4,327
North St. Vrain River	2,570	1,315	460 (8.45)	480 (11.07)	395 (8.22)	2,650

1/ Only a single cutthroat measured

the Yellowstone River more than 85 miles below the spray area contained DDT, and the metabolite, DDE, in their bodies. Fish taken two months after the spray had as much or more DDT in their bodies as fish taken less than one month after the spray. Rainbow and cutthroat trout taken from lakes contained high concentrations of DDT, compared with trout from streams. In general, whitefish bodies contained more DDT than did those of cutthroat, rainbow, or brown trout.

Additional fish specimens taken from affected waters in the winter and spring of 1957-1958 were forwarded to the chemist for analysis in June, 1958, and the results are awaited. Fish collected throughout the summer of 1958 are being held for analysis, should the latest results indicate the presence of DDT.

Effects of forest insect spraying on trout and aquatic insects in some Montana streams. Final Report. Processed Rept., U.S. Forest Service, 1958, pp. 1-56.

Grasshopper

Grasshopper control studies carried on by the Agricultural Research Service near Melville, Montana, in August,1958, were designed to test the effectiveness of aldrin, heptachlor,toxaphene, and malathion when applied by airplane to 640-acre plots. Observations were made by a biologist from this office to determine effects on fish. A small stream passed through some of the areas in such a way that three different toxicants could have been in the water at one time. Interpretation of causes of mortality was thus rendered most difficult.

It was concluded from the observations that little or no toxicity was sustained by longnose dace, brook trout, common sucker, or northern mountain-sucker in the section treated with aldrin in oil at the rate of two ounces per acre. In the section treated with toxaphene at 1.5 pounds per acre, the toxaphene or the toxaphene-aldrin combination from upstream eliminated all fish and aquatic invertebrates, and many garter snakes and spotted frogs were found dead after the spray. The section treated with heptachlor water emulsion was devoid of fish a week after the spray, and it is possible the toxicant may have driven the fish downstream rather than killed them in the area. Bottom organisms were not reduced in numbers by aldrin or heptachlor.

Fish-Pesticide Relationships

Under Public Law 85-582 the Secretary of the Interior is authorized and directed to undertake studies on the effects of pesticides on fish and wildlife resources. In the implementation of this work, an investigation called the Pesticide-Fish Relationships was established on November 18, 1958. The organization and development of the new program has received considerable attention by the Rocky Mountain Sport Fishery Investi-

gations. Part of the staff of the new studies will come from our Logan, Utah, headquarters, and the laboratory will be developed at Denver, Colorado. Much planning has gone into the furnishing of proper laboratory facilities at Denver, where the staff and equipment will handle chemical analysis, bioassay work, and other projects pertaining to the general problem.

In preparation

Benson, Norman G.
Report on limnological studies on Yellowstone Lake.

A note on fish mortalities associated with cloudy weather.

Bulkley, Ross V.
The use of branchiostegal rays to determine age of lake trout, Salvelinus namaycush (Walbaum), from Fish Lake, Utah.

Bulkley, Ross V.
Report on 1958 studies on the greenback trout.

Age of Yellowstone Lake cutthroat.

Cope, Oliver B.
Effects of spruce budworm spraying on fish in the Yellowstone River system.

Two parasites of cutthroat trout in Yellowstone Lake.

Cope, Oliver B. and Orville P. Ball
Mortality studies on cutthroat trout in Yellowstone Lake.

FISH DISEASE RESEARCH

Eastern Fish Disease Laboratory
Leetown (P. O. Kearneysville), W. Va.
S. F. Snieszko, Chief.

One of the most difficult steps in organization and operation of a research laboratory is selection of a well balanced staff. For research service in the field of fish diseases it is necessary to have persons trained in general pathology, bacteriology, virology, parasitology, histopathology, biochemistry, and fishery biology. It is very gratifying to report that Dr. Glenn L. Hoffman, a well trained and experienced parasitologist has been added to the Leetown staff.

Valuable information has been obtained on the absorption of chlortetracycline (aureomycin) by the trout liver and on the infectious nature of pancreatic necrosis.

Fish tissue culture technique has been advanced by obtaining serial transfers of tissues in vitro.

Fish tissue cultures were also successfully used for the cultivation of viruses isolated from fishes and for diagnostic purposes.

Erythromycin has been found to be the most promising drug for the control of kidney disease.

Further experimental evidence has been obtained on the relationship between ammonia and blue-sac disease.

Microhematocrit has been found to be a reliable and rapid method for detection of anemia in fish.

Extension activities have been improved by training and assignment of two new extension specialists and publication of 15 up-to-date leaflets on the most important fish diseases.

A permanent school for training in fish diseases has been established at Leetown.

Blood and tissue concentrations of antibiotics in fish. S. F. Snieszko and G. L. Bullock.

We have found in experiments performed and published during the past several years that chloramphenicol and oxytetracycline (terramycin) were effective in the treatment of fish furunculosis and ulcer disease, but chlortetracycline (aureomycin) had no therapeutic effect. This was baffling because in vitro and in higher vertebrates oxy and chlortetracycline have similar bacteriostatic and therapeutic spectrum. The manufacturers of aureomycin expressed considerable interest in our results.

In the first experiment antibiotics were given orally with food and were available to fish at the rate of 50 milligrams per kilogram of body weight per day. The blood levels were almost undetectable, but somewhat better with oxytetracycline. Higher concentrations were found in the muscle tissues, still higher in the kidney and the highest in the liver.

In another experiment each antibiotic was administered in a gelatin capsule as a single oral dose. Dosage was 100 milligrams per kilogram of fish. Within four hours oxytetracycline was found in the blood but was much higher in the liver. Chlortetracycline was not detectable in the blood but was present in the liver. Twenty-four hours after treatment both antibiotics were below their detection threshold in the blood but liver levels were even higher. After 2 and 3 days the liver level of oxytetracycline was declining faster than that of chlortetracycline which was still high. The higher blood levels of oxytetracycline shortly after administration may explain why this antibiotic was superior to chlortetracycline in the control of such systemic bacterial infections as fish furunculosis and ulcer disease.

Parasitology of fishes. G.L. Hoffman and student assistant William E. Purcell.

In February 1958, Dr. Glenn L. Hoffman joined the staff. Before coming to Leetown, Dr. Hoffman was a professor of parasitology at the Medical School of the University of North Dakota. He is especially interested in fish trematodes and is a recognized authority in his field. Upon his arrival he established and equipped the available space as his laboratory. In addition to his research he has participated in teaching activities and diagnostic services.

A new species of strigeoid trematode metacercaria has been found in fallfish, creek chubs, common shiners, and the cutlips minnow. The worm was reared in baby chicks. It is most nearly like Neogogatea pandionis Chandler and Rausch, 1948, from the osprey. Eggs of this new worm were obtained from experimentally infected baby chicks and incubated in petri dishes. Normal miracidia hatched from the eggs in 27 days which is considerably longer than the 10 days required for Posthodiplostomum, Diplostomum, etc.

A blood flagellate (Cryptobia) was found in fallfish during the summer months. It could not be found again in October and November and it disappeared from the blood of fallfish collected in September. Attempts to release the possible latent infection by injecting cortisone daily for 7-11 days was successful in 2 out of 4 attempts. Raising the water temperature from 55° F to 72° F did not release the possible latent infection. Numerous attempts to culture the organism failed.

An unusual parasitic disease has come to our attention from Three Rivers, Quebec. Very high mortalities resulted from tapeworm larvae in the heart and liver of fingerling trout which were fed copepods recovered from a water supply filter. The tapeworm has not been identified yet, but there is little doubt that the fish became infected from eating the infested copepods.

Preliminary work has been done on white blood cell differentials of trout in the hope that this can be used to study the effects of parasites of fish.

Pancreatic Necrosis. S.F. Snieszko, Ken Wolf, C.E. Dunbar, J.E. Camper and L.L.Pettijohn.

Pancreatic necrosis is a disease of fry and young fingerlings of salmonid fishes, brook trout in particular. It is characterized by spiralling of the diseased fish. The intestinal tract is free from food and pathological changes can be detected in the pancreatic tissue and in the striated muscles. Evidence at hand indicated that it is an infectious disease, probably caused by a virus, but an initial experimental proof was still needed. Therefore experiments were made to clear this point.

Eyed brook trout eggs were received in November 1957 from Bellefonte, Pa., Berlin, N.H., Walhalla, S.C., and Beaver Creek, Md. A spontaneous outbreak of pancreatic necrosis occurred in the fry hatched from the Bellefonte, Pa. eggs. This was the fourth season that this disease appeared, without artificial infection, in fry hatched from the Bellefonte eggs and spread to brook trout fry hatched from other sources (when such were present at Leetown). Brook trout fry from Berlin, Walhalla and Maryland hatcheries were divided and some were infected with material obtained from fish with pancreatic necrosis. In the infected lots typical pancreatic necrosis developed, with mortalities ranging from 70 to 80 percent. Control trout remained free from this infection during the experiments. In trout fed with brine shrimp larvae the disease had a more acute course. However, trout fed brine shrimp were more uniform in size and better in general appearances. Addition of iodine or iodine containing synthetic thyroid hormone (Cytomel) had no visible effect.

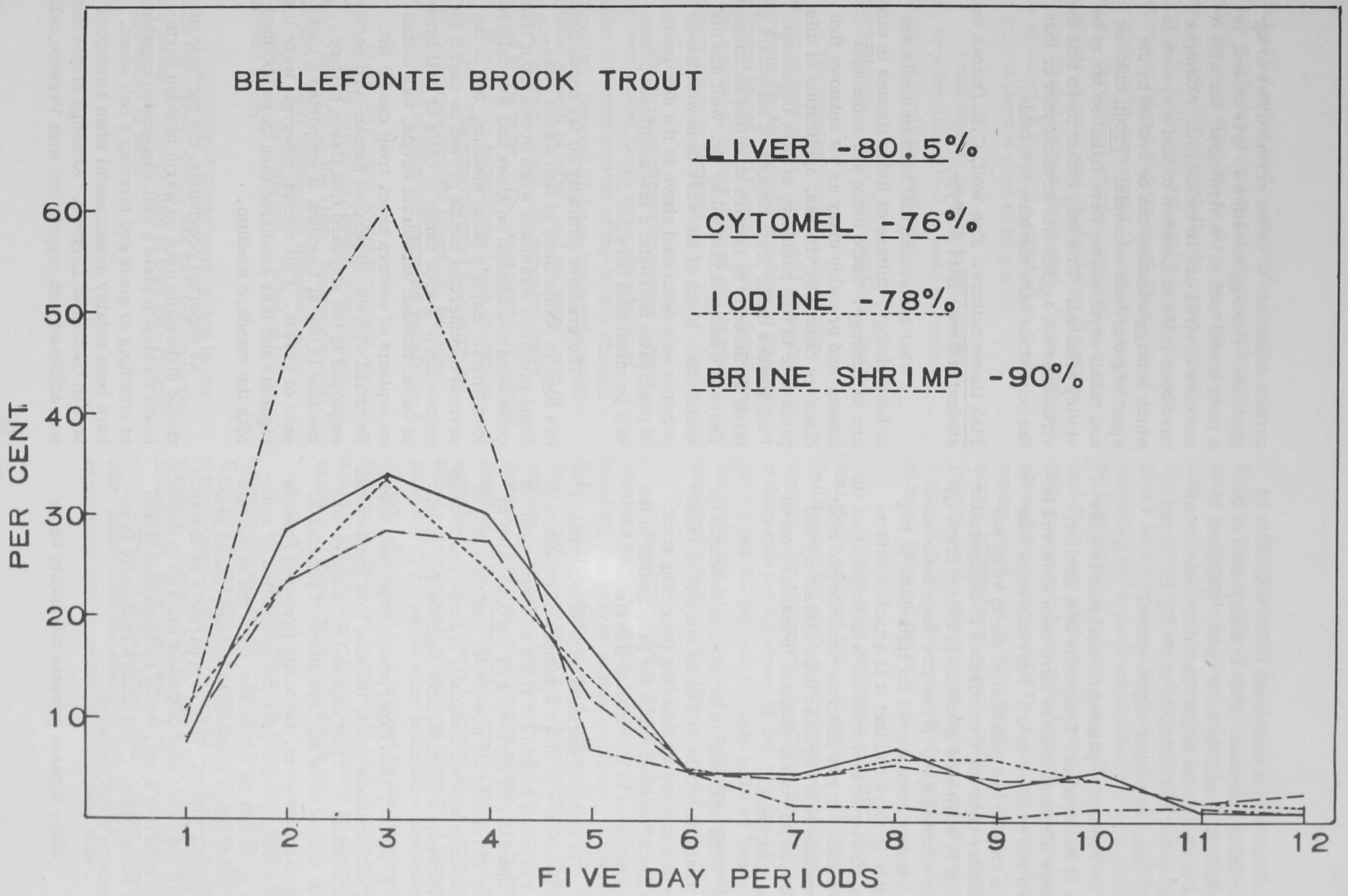
Results of these experiments are presented in detail in several graphs. In the figure are presented mortalities per five-day periods in brook trout fry and fingerlings from Bellefonte. This was a spontaneous outbreak. Enrichment of the liver diet with two sources of iodine (iodine and Cytomel) had no effect. If the liver diet was replaced by nauplii of brine shrimp, pancreatic necrosis had a more acute course with higher total mortalities.

Fry which hatched from eggs received from Berlin, N.H. were very small. If beef liver was fed many were not able to take food, changed to "pinheads", and gradually wasted away. Such fry exposed to infection with pancreatic necrosis, did not show the presence of the disease. Another batch of the same fry were fed brine shrimp larvae and they grew normally and uniformly with no pinheads. Berlin brook which were fed brine shrimp trout were exposed to infection with pancreatic necrosis at the same time, and in the same manner as the liver-fed fish were exposed. Pancreatic necrosis ran an acute course in the infected and brine-shrimp-fed brook trout from Berlin.

Brook trout fry hatched from eggs received from Beaver Creek State Hatchery in Maryland and Federal Hatchery at Walhalla, South Carolina have grown well when fed beef liver. When both lots of fry were exposed to infection with pancreatic necrosis they soon displayed typical symptoms of this disease. In both lots of fry the disease had the typical course in infected fish. Controls were free from symptoms of pancreatic necrosis.

True viruses can develop and multiply only inside of living cells. Therefore experiments with viruses performed in vitro require cultures of living tissues. Some viruses if introduced into a tissue culture of the right type cause clearly visible cytopathogenic effect. If a virus is cytopathogenic to a tissue culture, research on such a virus is greatly facilitated. This is also one of the most reliable and the fastest methods for the isolation and demonstration of the presence of cytopathogenic viruses in animals.

Experiments in the spring of 1958 with inocula prepared from fish presumptively diagnosed as having the disease have, in every instance, evoked degeneration of epithelium from cultures of adult trout tissues. Storage of typically affected fry at 20° C for 3 years or in 50% glycerol at 4° C for 1 year did not eliminate the degenerative factor present in inocula prepared from such fish. Hour-long treatment of inocula at 60° C destroyed the ability to evoke degeneration, and treatment with ether reduced it. Plasma from adult brook trout inhibited degeneration indicating possible presence of antibodies in older fish. One lot of inoculum in its sixth transfer


BELLEFONTE BROOK TROUT
LIVER -80.5%
CYTOMEL -76%
IODINE -78%
BRINE SHRIMP -90%
PER CENT
60
50
40
30
20
10
1
2
3
4
5
6
7
8
9
10
11
12
FIVE DAY PERIODS

was effective at a calculated 1/300, 000, 000th of the original material. This is interpreted to be propagation of an infectious agent. Cultures of HeLa cells (human origin) inoculated with identical material and incubated at 19° C, 25° C and 37° C failed to exhibit degeneration.

Typically, pancreatic necrosis is a disease of brook trout. Symptoms of a similar, perhaps identical disease have been observed in brown and rainbow trout. Previous work with tissue culture has established the in vitro susceptibility of brook trout tissues to degeneration evoked by a filtered inoculum prepared from typically affected fish. It has now been determined that rainbow trout caudal fin epithelium is susceptible in vitro, and that it is attacked with results similar to that obtained with brook trout fin epithelium. In two different trials brown trout fin epithelum in vitro has been refractory and evidenced no visible reaction to inocula proven to be infectious.

The next step to be taken in research on pancreatic necrosis will be an attempt to reproduce this disease by infecting fingerling trout with inoculum obtained by the propagation of the infective agent in cultures of fish tissues.

In order to perform such an experiment it is necessary to make a number of transfers in vitro in order to find out if the etiologic agent of this disease can reproduce in vitro, and also in order to remove the possibility that the agent of the disease was mechanically transferred through a fish tissue culture without reproducing in it.

So far, such experiments have been made only with rainbow trout, because this species was first available. Trout exposed to infective material did not show any symptoms of pancreatic necrosis. However, inoculum prepared from infected trout and used for the inoculation of a culture of caudal fin epithelium from the same rainbow trout and of brook trout evoked typical cytopathogenic effect. This is apparently an instance where the intact host displayed marked resistance to an infective agent, while isolated host tissue was susceptible. This occurs frequently in research on virus diseases.

Many viruses possess the ability under certain conditions to cause erythrocytes to agglutinate. Hemagglutination as it is called, is a most useful tool to the virologist, for with it amounts of virus can be measured. Attempts have been made at Leetown to find a system in which hemagglutination can be evoked by the agent of pancreatic necrosis. Trout, chicken and rabbit erythrocytes have failed so far to be visibly affected. This may either mean that the virus does not agglutinate erythrocytes or that the conditions have not been favorable.

Fish tissue culture. Ken Wolf, C.E.Dunbar and student trainee, Earl A. Pyle.

What bacteriological culture media are to bacteriology, culture of living tissues in vitro are to virology. Since there are some well known, and probably many as yet unknown fish diseases caused by viruses, application of this procedure in fish pathology is very important. Fish tissues differ from tissues of mammals and birds sufficiently to require modified techniques. These are being developed by Dr. Wolf and his assistants. Much of the work done with tissue cultures was described above in the discussion of pancreatic necrosis; a few additional points will be mentioned here.

The greatest difficulty so far encountered has been to obtain lines of fish tissue cells. Some progress was made when brown trout tissue cells were established on glass and sub-cultured four times. Eagle's basal medium, one of the several commercial media proven so useful for cultivation of a wide range of cells or cell lines of warm-blooded vertebrate origin, was tested on explants of eastern brook trout caudal fin. Fortified with the 20 percent human cord serum employed in the standard fish tissue culture medium in use at Leetown, it supported a measure of growth. Cell sheets, however, were less vigorous and less extensive than those obtained with the standard medium.

As reported previously, the methods of tissue dispersion used on warm blooded vertebrate tissues to yield a cell suspension capable of attaching to glass and forming a cell sheet, have been notably unsuccessful when attempted on fish tissues at Leetown. Although dispersion was achieved with trypsin and with Versene, cells

which were separated were not inclined to attach to glass and had unsatisfactory viability. Until recently attempts to disperse arthropod tissues have had essentially the same results. A group in California effected a break-through with insect tissue and successfully achieved dispersion with an extract of snail hepatopancreas. Through their kind cooperation a supply of this extract was obtained and digestion of trout caudal fin was attempted. Some dispersion resulted; a few cells attached to glass, but for too short a time to be useful.

Trial digestions with hyaluronidase have thus far also failed to yield an entirely satisfactory cell suspension.

Kidney disease. Ken Wolf and C.E. Dunbar.

The economic importance of this disease is very great. It is still spreading throughout the hatcheries of salmonid fishes in the United States and Canada, and experiments on the control of kidney disease are of great and immediate importance.

Thirty-four chemotherapeutic agents have been tested for in vitro effectiveness against different strains of the kidney disease bacterium. Several of these drugs effectively inhibited growth in vitro, and six were used in the first therapy trials with experimentally infected brook trout. The most promising drugs were selected for experiments with fingerling brook trout. The experimental fish were infected by intraperitoneal injection, because other methods of infection were not satisfactory.

In the first experiment treatment started when the daily sampling showed development of specific bacteria in the kidney of experimental trout which were infected by injection of the pure culture of the pathogen. Period of treatment was 21 days.

Six additional drugs were tested in the second experiment which was carried out in the same manner. The results of both experiments are presented in table 1.

Erythromycin-fed fish which survived kidney disease infection were killed 206 days after treatments had been stopped. Experimental and control lots were examined by stained kidney smear and by inoculation of kidney material into a bacteriological medium. Two of 51 fish surviving the experimental infection were found by both methods to have kidney disease. Control fish were negative, but nearly all fish of both groups exhibited the idiopathic granuloma (described as mycosis-like granuloma) common among older fish at this station. Although nearly 40 percent of the treated fish died, these results can be interpreted as being Erythromycin cures of at least some cases of experimentally-induced kidney disease.

The results presented in table 1 show that there are two drugs of choice. Gantrisin (sulfisoxazole) gives a very good temporary control. Erythromycin given at a rate of 100 milligrams per kilogram of fish per day (4.5 grams per 100 pounds) not only reduced losses, but under the experimental conditions apparently produced a permanent cure in some trout.

Brook trout from four hatcheries were obtained as eggs during the winter of 1957-58, hatched, and reared at this laboratory to investigate relative resistance of different strains. During June, they were inoculated with the kidney disease organism. One trough of each strain served as control; two troughs of one strain, and three troughs of remaining strains were used for inoculated fish. Growth from the seven strains of kidney disease was pooled and inoculated intraperitoneally. Two weeks after inoculation ten additional fish, which had been marked by fin clipping and allowed to heal, were added to each trough to test again the difficult-to-transmit nature so characteristic of this infection in brook trout.

The specific bacteria were first found in kidneys two weeks after inoculation, and the first mortality due to kidney disease began about a week later. Mortality among all strains rose steadily, reached a peak about five weeks after inoculation, then subsided. A second rise in mortality occurred during September. Three months after inoculation the status of fish in this experiment was as shown on top of next page.

Origin of Eggs	Av. Wt. at Start [1/]	Percentage Mortality	Marked Fish
Bellefonte, Penna. (State)	6.3 gms.	89.6%	No mortality
Beaver Creek, Md. (State)	12.7 gms	93.0%	No mortality
Walhalla, S.C. (Fed)	9.3 gms	98.0%	No mortality
Berlin, N. H. (Fed)	14.5 gms	87.0%	No mortality

Table 1.--Combined results of two experiments on the treatment of kidney disease in fingerling brook trout.

Drugs	Dosage Milligrams per kilogram of fish per day	Percentage of losses from start up to One month After treatments were stopped.	Three month	Six month
Erythromycin	40	54		
Erythromycin	100		11	40
Carbomycin	40	65.5		
Tetracycline	40	64		
Novobiocin	40	64		
Furacin	100	78		
Furoxone	100	63.5		
Gantrisin	200	1 .5		
Gantrisin	200		74	
Phenoxyethanol	200		78	
MK-51(2-acetylamino-5-nitrothiazole)	40		65	
Sodium pencillin G	75		59	
Kynex (sulfamaethoxy-pyridazine)	200		39	
Inoculated Controls I		62		
Inoculated Controls II			83	
Not inoculated controls		0		

Half a year after the fin-clipped fish were added to trout with experimental kidney disease, 10 percent of them died with symptoms of this disease. In a previous experiment, only fish with external abrasions became infected with kidney disease. It is possible, therefore, that in this case fin clipping contributed to the infection.

A very large scale experiment on the transmission and control of kidney disease was performed in 1957 at the Federal Hatchery at Berlin, N.H. A large number of data was accumulated which await evaluation. A new experiment is in progress. Mr. Henry Delisle, and Mr. Dan McKinnon, hatchery manager and assistant hatchery manager devoted much time to make these experiments successful.

Some stock cultures of the kidney disease organism have been maintained on Mueller Hinton medium (Difco) with 0.1% L-cystein HLC for 17 months.

Blue-sac disease. Ken Wolf.

In a book on fish diseases by Reichenbach-Klinke (1957) it is stated that blue-sac disease is caused either by bacteria or inheritable constitutional disorders. Dr. Wolf's research has implicated the products of fish egg metabolism as the most likely cause of this pathological condition. Additional experiments were performed in the winters of 1957 and 1958.

A small scale experiment was made to determine the effect of unfavorable shipping experience on the incidence of blue-sac disease. Twenty-day-old brook trout eggs were subjected to simulated shipment for a period of 4 days. Paired controls were kept in water and incubated under standard procedures. Eggs subjected to simulated shipment were held on a screen floor in plastic vessels. Ice was continually kept on a

perforated drip container above the eggs in one pair of vessels. In another pair ice was placed above the eggs for only about 5 hours of each day. The last pair was kept in a moist chamber at 54°F. but was not iced. Average blue-sac disease incidence for replicate lots was as follows:

Controls--maintained in water	18.0%
Ice always present above "shipped" eggs	20.4%
Ice above "shipped" eggs 5 hrs./day	22.0%
No ice above "shipped" eggs (T.54° F)	38.1%

These results indicate that a significant increase in blue-sac disease occurred in fry from eggs which were not iced during a simulated shipment of 4 days. A similar experiment with trout eggs did not yield conclusive results.

Another experiment was made in order to determine the effect of ammonia and urea on developing eggs of eastern brook trout and the incidence of blue-sac. Eyed eggs were exposed in special containers, 30 eggs per container, and in triplicate, to graded increases of ammonium hydroxide, urea, or both. Some eggs were incubated in a large container with the same water at the same temperature and otherwise similar conditions, except that the water was static.

In the early stage of the experiment the incidence of blue-sac was significantly higher when eggs were exposed to ammonia; urea had no such effect. Differences in the degree and incidence of blue-sac became insignificant near the end of the experiment (table 2). Chemical tests have shown that in a closed egg incubation system, ammonia, but not urea, have gradually accumulated. Static water conditions were wholly unsatisfactory for trout egg incubation. Most of the eggs died and the few fry which hatched had blue-sac.

Hematological examination of trout blood. S. F. Snieszko with assistance of the following trainees: J. E. Camper, L. L. Pettijohn, and Fred. J. Howard.

In some fish diseases and in some types of malnutrition fish become anemic. Detailed study of anemia requires employment of numerous hematological procedures. However, in routine examinations for anemia only red cell counts and hemoglobin determinations are made. Red cell counts can be considered reliable only if done by persons experienced both in the technique and evaluation of the results. Since red cell counts are time consuming, only relatively small fish sample can be examined from a fish population. Clinical pathologists consider hematocrit as the most useful for routine detection of anemia. Since the recent development of the microhematocrit technique, determination of hematocrit became possible even with the small fish found in fish hatcheries.

At the time of the preparation of this report microhematocrits have been determined for several hundred fingerling trout, but the results have so far been evaluated for a relatively small part of the total fish examined. The mean figures so far obtained are shown on top of next page.

Histopathology:-- C. E. Dunbar and Dr. G. L. Hoffman

One of the most reliable procedures in diagnosis of diseases is histopathology. Unfortunately this phase of work on fish diseases is grossly understaffed and underdeveloped in some of the fish pathological laboratories.

Mr. Dunbar has shown interest and skill in histopathological methods and a laboratory is being established and equipped for this work. Recently a modern autotechnicon was added. Evaluation of the histological slides prepared from the organs and tissues of diseased fishes will be improved by exchanging slides and information with the Willard Laboratory and with other histopathologists interested in fish. There is a large backlog of material to be examined.

Training activities:--All staff members.

During the past 18 months two fish culturists completed one 12-month in-service training course and another 12-month course for three trainees is in progress. A two-week short course in fish diseases was given at the laboratory for 12 students. Trainees assigned on an annual basis serve as part-time laboratory assistants.

Trout Species	Hemoglobin as determined in A.O. Hemoglobinometer (grams per 100 ml of blood)	Red cell counts per cubic millimeter	Micro-hematocrit
Brook trout	9.7	1,260,000	49
Brown trout	8.1	1,180,000	44
Rainbow trout	8.6	1,370,000	53

Table 2.--Average percentage blue-sac disease [1/] occurring in groups subjected to different conditions during incubation.

Time of observation	Conditions to which subjected during incubation				
	Control	Ammonia added	Urea added	Urea and ammonia added	Closed system
Completion of hatching	26%	57%	18%	58%	41%
4 days after hatching	66%	94%	50%	97%	94%
Termination - 9 days after hatching	93% [2/]	100%	86% [2/]	98%	91%

[1/] Fry with a volume of fluid equal to or exceeding Stage I blue-sac disease.
[2/] Severity of blue-sac disease in these groups was significantly less than in the others.

Extension services.-- R.G. Piper, L.L. Pettijohn and J.E. Camper.

Extension services include diagnostic services, and dissemination of information on diagnosis and control of fish diseases. During the second half of 1957 and the first half of 1958 Mr. Piper was performing the diagnostic services for Bureau Regions 3, 4, and 5. During the second half of 1958, Mr. Piper was transferred to LaCrosse, Wisconsin, and Mr. Camper went from the training school to Pisgah Forest, N.C. hatchery. Mr. Pettijohn assumed Mr. Piper's duties at Leetown for the northeastern states. The Eastern Fish Disease Laboratory retains technical liaison with these hatchery biologists and one Leetown biologist will visit hatcheries in each of the three regions once each year.

One of the most important achievements in the extension field was preparation and issuance of 15 new leaflets on fish diseases by laboratory staff members.

The summary of diagnostic services performed during the past 18 months is presented in table 3.

Table 3.--Diagnostic Services

Number of examinations performed	125
Number of hatcheries visited	38
Diseases diagnosed	
Unknown or doubtful	29
Bacterial gill disease	15
Columnaris	8
Furunculosis	10
Pancreatic necrosis	10
Nutritional gill disease	3
Kidney disease	9
Sunburn	5
Peduncle disease	2
Fin rot	2
Infectious dropsy	6
Toxic agents in water supply	7
Blue-sac	1
Gas bubble disease	1
Nutritional deficiency	5
Saprolegnia or other fungi	2
Different animal parasites	8
General Information	2
	125

Mss. in preparation

Hoffman, Glenn L.

Recommended Treatment for Fish Parasite Diseases.

Key to the Major Groups of Fish Parasites.

Studies of the life cycle of Apatemon gracilis pellacidus (Yamag.)

Trematoda - Strigeoidea.

Dunbar, Clarence E.

A case of sunburn in fingerling rainbow trout.

Piper, R. G. and Ken Wolf

Cost of disease treatment.

Snieszko, S. F., Jimmy E. Camper and Lyle L. Pettijohn

Infectious nature of pancreatic necrosis.

Snieszko, S. F., C.E.Dunbar and G.L.Bullock

Resistance to ulcer disease and furunculosis in eastern brook trout.

Wolf, Ken

Plasmoptysis and gelation of erythrocytes in coagulation of blood of freshwater bony fishes.

Wolf, Ken and C. E. Dunbar

In vitro and in vivo drug-sensitivity of the bacterium of fish kidney disease.

Western Fish Disease Laboratory
Seattle, Washington
Robert R. Rucker, Chief

The Western Fish Disease Laboratory is concerned primarily with basic research on infectious diseases of salmonid fishes. Personnel and facilities are available for detailed study of viral, mycotic, bacterial, and parasitic diseases of wild and hatchery-reared populations of salmon and trout. Additional to basic studies, the laboratory extends diagnostic services to allied Federal, State, and private agencies upon request.

Research efforts over the past eighteen months have been directed upon four major areas: acid-fast bacterial disease; red mouth disease; virus-like diseases; and an examination of the potential of gross parasites as racial indices. Details of progress in these areas of research are reported below.

A closely integrated external research program was initiated in early 1957 by contract arrangements with Washington State Department of Fisheries, Oregon State Fish Commission, and the University of Washington. The purpose of these contracts was to effect a long-term, collaborative survey and research program on diseases of the important Columbia River stocks of salmon and steelhead trout.

An in-service training program of ten months duration was organized in the fall of 1957 to provide additional training in fish disease diagnosis and management to selected hatchery personnel. To date, two trainees have completed the program and two others are currently in training.

Organization and Personnel

Several new personnel were added to the staff during the reporting period. The new appointments have served to broaden research activity and to fill existing vacancies.

Mr. Thomas J. Parisot, M.S. (Bacteriology) was appointed in July, 1957, to assist in bacteriological studies and to conduct research on virus-like diseases of fish and methods of fish tissue culture.

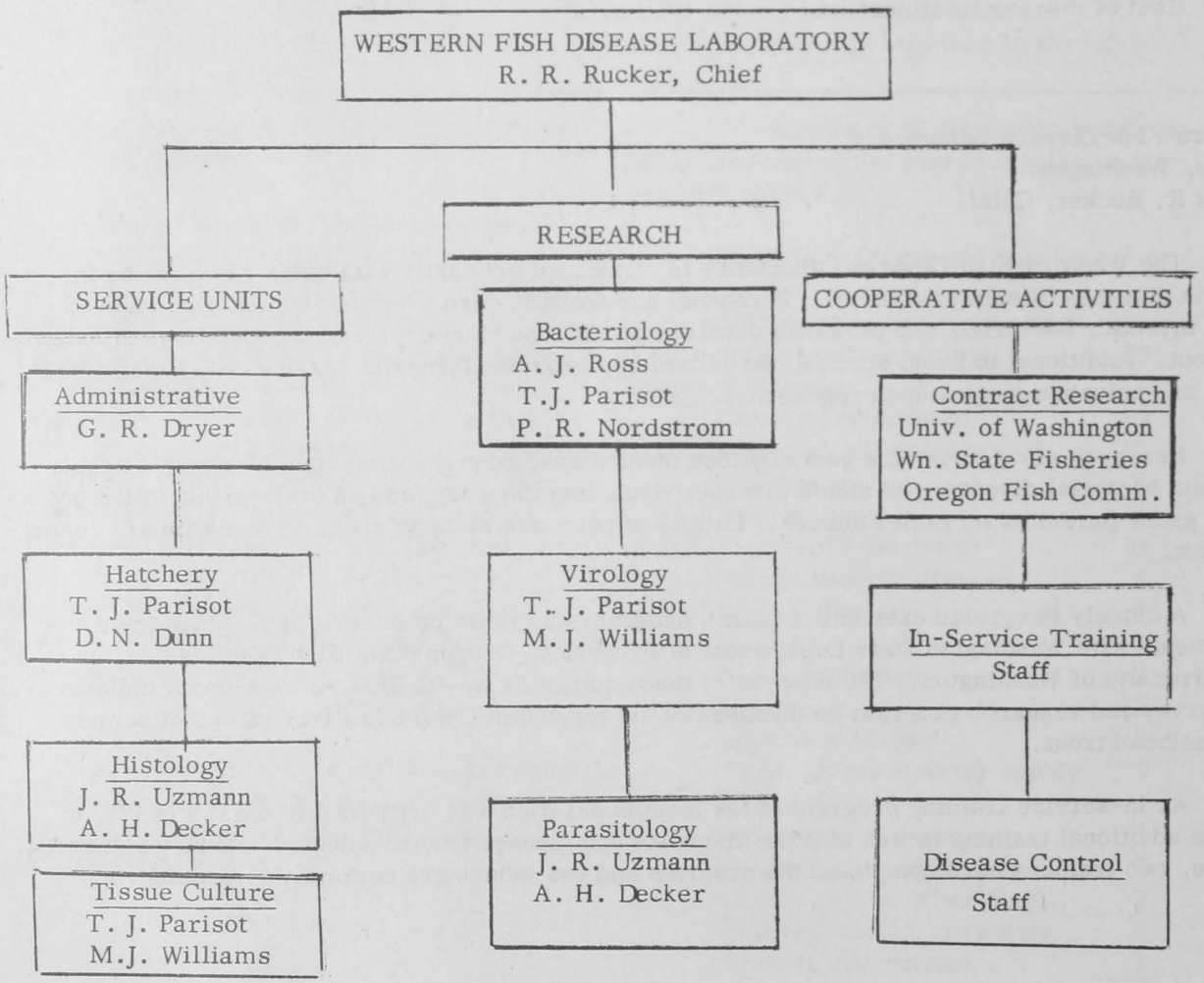
A histology unit was organized in early 1958 to provide more complete diagnostic and research services within the laboratory. Miss Aletha Decker, B.S. (Zoology), was appointed in March, 1958 to provide this service and to assist in parasitological studies.

Miss Phyllis Nordstrom, B.S. (Bacteriology) was appointed to the staff in May, 1958 to assist in bacteriological research.

Mrs. Gail Dryer, Clerk-Stenographer, joined the staff in July, 1958. She brings with her a background of ten years experience in Service affairs.

Miss Miriam Williams, B.S. (Bacteriology) was appointed in December, 1958 to assist in development and maintenance of the newly-developed tissue-culture unit.

A summary of present laboratory organization, functions, and responsible individuals is presented below.



Research Projects

Acid-fast bacterial disease:--A chronic, infectious debilitating disease of salmon and rainbow trout, including the sea-run or steelhead form. This disease has been a subject of intensive investigation since 1955 when the first of a number of strains of acid-fast bacteria was isolated from mature, chinook salmon at the Spring Creek, Washington, Federal hatchery. Extensive survey studies have since revealed widespread distribution of mycobacteria in salmon and steelhead trout returning to Washington, Oregon, and California hatcheries. In direct contrast, however, seaward migrants and adult salmon from natural spawning areas were rarely found to be infected. A significant feature of the disease is the tendency of infected fish to return from the sea as immature adults or in a condition of sexual under-development. In either case, the reproductive potential of such fish is lost to the normal cycle. Recent observations at the Coleman, California hatchery are illustrative: approximately 4 percent of the chinook salmon spawned over a two-day period were abnormal in size or brightness. Among those fish, 72 percent showed positive evidence of Mycobacterium infection in appropriately stained liver smears, and 70 percent were sexually under-developed. Only 2 percent of a random sample of normal appearing fish was similarly infected. Recurring evidence indicates that the inclusion of raw salmon products in hatchery diets is the principal mode of transmission of the disease among hatchery-reared fish. Unless this practice is curtailed, or modified effectively, the incidence of the disease seems likely to increase with time.

The cultural characteristics of several different isolates have been studied intensively. It now appears certain that at least two different species of Mycobacterium are prevalent. Species and strain differences among the various isolates have been examined by biochemical and immunological methods. Homologous agar diffusion precipitin tests using rabbit antisera have revealed the presence of several antigen fractions. The studies will be expanded to include comparisons between the mycobacterial isolates from salmonids and those available from other fishes, cold-blooded vertebrates, and soils. Heterologous antigen-antibody agar diffusion precipitin tests have been designed for determination of affinities between these various mycobacteria.

Chemotherapeutic studies were designed and executed in an attempt to work out effective treatment and control of mycobacterial disease. Fifteen mycobacteriostatic drugs were tested in vitro against six different Mycobacterium isolates from salmon and steelhead trout, and four additional isolates from non-salmonid fishes. Results were equivocal, at best, and it would appear that chemotherapy with presently available drugs is not practical. For the present, at least, it is believed that careful fish-cultural practices may be sufficient to minimize or eliminate the disease wherever present.

Four manuscripts were prepared on the significance, identification, and detection of acid-fast bacterial disease.

Redmouth disease:--A chronic bacterial disease of young rainbow trout characterized by the presence of a pseudomonad-like organism. Investigations of this disease have been made intermittently over the past several years, but until recently no significant progress was made. The disease may be considered a bacteremia with gross symptoms including lassitude, exophthalmus, and typically, pronounced extravasation or "reddening" of the mouth, pharynx, and branchiostegal regions.

The disease is unusual in many respects and has been a recurring problem for many years in the Snake River region of Idaho and in certain areas of California. Wherever present, the disease has produced significant, but not catastrophic losses of under-yearling rainbow trout. Treatment, for lack of better knowledge, has been largely symptomatic, and has employed the use of sulfa drugs and management prophylaxis with varying degrees of success. Yearling and older fish appear to be more or less immune to the disease and rarely show gross symptoms; however, it is now evident that such fish are carriers and may readily transmit the disease to the more vulnerable juveniles.

The apparent etiologic agent of the disease, a pseudomonad-like organism, was recently

isolated from diseased rainbow trout at Hagerman, Idaho. Typical red mouth disease was experimentally produced in fish at the Seattle laboratory from the Hagerman isolates. The agent of the disease, a gram-negative, lophotrichic organism, was repeatedly recovered from experimental lots of fish. The disease is reproducible in 2 - 3 days following intraperitoneal inoculation, or in approximately 10 days following constant exposure to a drip suspension of organisms.

With the etiologic agent at hand, rational chemotherapeutic studies may be conducted. A preliminary survey of 29 drugs has been completed. Several antibiotic, sulfonamide, and nitrofuran compounds have shown promise in plate growth inhibition tests. Tube-dilution studies are projected to ascertain optimal effective drug concentrations prior to in vivo trials.

Tissue sections of infected fish have been prepared for interpretation of the histopathology of the disease.

Virus-like diseases:--A complex of acute diseases of young salmon of unknown etiology involving one or more filterable agents. First recognition of a virus-like disease in salmon occurred in 1951 when an outbreak of disease occurred at the Leavenworth, Washington hatchery. Extensive studies of diseased fish revealed the presence of a filterable pathogen and a histopathology picture comparable to that observed in moribund fish from the Winthrop, Washington station in 1950. The use of raw salmon products in production diets was determined a source of infection and little trouble was experienced after diets were appropriately modified. A new outbreak of this disease was recognized at the Winthrop station in the spring of 1958. Pathogenicity studies and histopathology findings confirmed the original diagnosis. It was concluded that the source of infection in this case was from naturally spawning salmon in the river system above the hatchery water intake.

In the present state of knowledge, this disease is recognized as a distinct entity among the virus-like diseases of salmon. Studies will be continued on an intermittant basis in an attempt to isolate and characterize the etiologic agent. A summary review of findings to date has been prepared for publication.

Since its establishment in 1943, the Coleman, California salmon hatchery has annually experienced significant losses of fingerling chinook salmon. Preliminary investigations conducted in the spring of 1957 related the losses to the occurrence of a filterable, infectious disease agent. Subsequent histopathology studies disclosed pathologic changes in various organs of affected fish with nuclear and cytoplasmic inclusions indicative of virus infection. These observations were confirmed in 1958 following a recurrence of the disease. Typical symptoms of the disease were produced experimentally in chinook salmon fingerlings at the Seattle laboratory. Epidemiology studies have included a survey of past outbreaks, treatments, water conditions, diets, possible indigenous vectors, and hatchery management of the disease. Field experiments have been initiated to determine the influence of varied environmental factors and cultural practices in relation to incidence of the disease.

A similar, possibly distinct, disease has been observed in chinook and silver salmon fingerlings from four hatcheries in the lower Columbia River region. The disease has been studied briefly from tissue sections only pathologic changes of the liver and pancreas accompanied by discrete, uniform nuclear inclusions are suggestive of yet another virus-like disease.

Parasitology:--More than 3,000 mature chum salmon from Asian, American, and high seas areas were examined for parasites as part of the International North Pacific Fisheries' Commission research program. The purpose of the study was to determine the utility of parasitological criteria for discrimination between Asian and North American salmon in high seas mixing areas. Thirty species of parasites were identified and studied with special regard to their distribution patterns, but none has proved to be a reliable index of continental origin.

Studies of similar nature were conducted with downstream migrant blueback salmon from the principal spawning areas in the Columbia

River system. Samples of approximately five hundred fish were collected from each of three areas during the course of the 1958 out-migration. These fish were examined in detail to determine their characteristic parasite fauna. Aside from a number of parasites common to all, certain pronounced differences were observed. Those fish originating from the Okanogan River system were distinguished by high rates of infection with Ergasilus sp., a parasitic copepod; those from the Salmon River system were characterized by similarly high rates of infection with a trematode parasite of the eye, Diplostomulum sp.; outmigrants from the Wenatchee River system were free of any exclusive mark. The sole remaining area producing a significant contribution of seaward migrants is Suttle Lake, Oregon, a tributary of the Deschutes River. Here, all migrants observed over a three year period were infected with the intestinal parasite Neoechinorhynchus sp. Thus, it appears feasible to formulate methods for determining relative abundance and survival estimates for these races in downstream mixing areas. A preliminary report of these findings including a rationale on statistical treatment of infection rate data was prepared for publication.

Contract Research

The University of Washington under contract research has continued the study of columnaris disease of fish in the Columbia River system. It has been determined that water temperature is a critical factor in the pathogenicity of columnaris disease. As temperatures increase, ordinary strains of Chondrococcus columnaris (the bacterium responsible for columnaris disease) become more and more effective in attacking and killing fish, but in addition, new and more deadly strains of C. columnaris appear. On the basis of serological studies which have been carried out it is possible to conclude that these new and deadly strains are mutants derived from ordinary strains of C. columnaris. These mutant strains are characterized by their ability to kill young salmon in less than 24 hours when introduced into the water in which the fish are held. High virulence strains of C. columnaris reappeared in the Columbia River system during the summers of 1957 and 1958 after an apparent absence of several years. Although it has been possible to carry out tests of virulence with only a fraction of the strains isolated, the available evidence obtained so far indicates that the high virulence strains of C. columnaris isolated in 1957 originated in the McNary pool, or in the warm water tributaries, i.e., the Snake or Yakima Rivers.

The Washington State Fisheries Department and the Oregon Fish Commission collaborated closely with this Laboratory to effect a comprehensive survey of bacterial diseases prevalent in adult salmon and steelhead trout returning to Columbia Basin hatcheries. For diagnostic purposes, several thousand liver samples were collected and preserved during the 1957 and 1958 spawning seasons. The samples were individually examined by bacteriological methods and the types and degree of bacterial infection were determined and catalogued. Results of the two year survey are being tabulated and analyzed and a joint-agency summary report is currently in preparation.

In preparation

Parisot, T. J.
Tuberculosis of fish: I I. Further contributions to the study of the acid-fast bacillus disease in salmonoid fishes. Identification of diseased fish. To be submitted to Prog. Fish Cult.

Parisot, T. J. and A. H. Decker
A comparative study of the staining characteristics of the causative agent of a mycobacterial disease of salmonoid fishes with the Fite-Faraco and Ziehl-Neelsen techniques. Submitted to Am. Rev. Tuberculosis and Pulmonary Diseases.

Ross, A. J.
A new species of Mycobacterium isolated from salmonoid fishes. To be submitted to Am. Rev. Tuberculosis and Pulmonary Diseases.

Ross, A. J., and F. P. Brancato
Mycobacterium fortuitum Cruz from the tropical fish, Hyphessobrycon innesi. To be submitted to Journal of Bacteriology.

Westgard, R. L.
A procedure for the detection of acid-fast bacteria in fish: a comparative study of digest concentrate vs. smear technique. Contract research contribution; manuscript prepared for publication.

FISH NUTRITION RESEARCH

Eastern Fish Nutrition Laboratory
Cortland, New York
Arthur M. Phillips, Jr., Chief

The research of the Cortland Laboratory is concerned with the nutrition and physiology of trout. Results of studies upon fat, protein, carbohydrate, and mineral metabolisms and the vitamin and mineral requirements of trout are for application in the preparation of diets for hatchery fish, resulting in increased growth, lower production costs, and reduced fish mortalities. An understanding of nutritional and physiological requirements of trout permits the development of new feeding techniques that reduce the cost of food storage, food preparation, and labor of feeding fish. The State of New York and Cornell University are cooperators in the laboratory program, through a long standing cooperative agreement.

The program of this laboratory the past eighteen months has consisted of five main projects. These projects have continued the stated aims and objectives of the Cortland Laboratory. These projects are:

1. The vitamin requirements of lake and rainbow trout and Atlantic salmon.

2. The establishment of normal blood composition for 16 organic compounds. In addition some of the factors effecting the daily fluctuation of blood glucose were investigated.

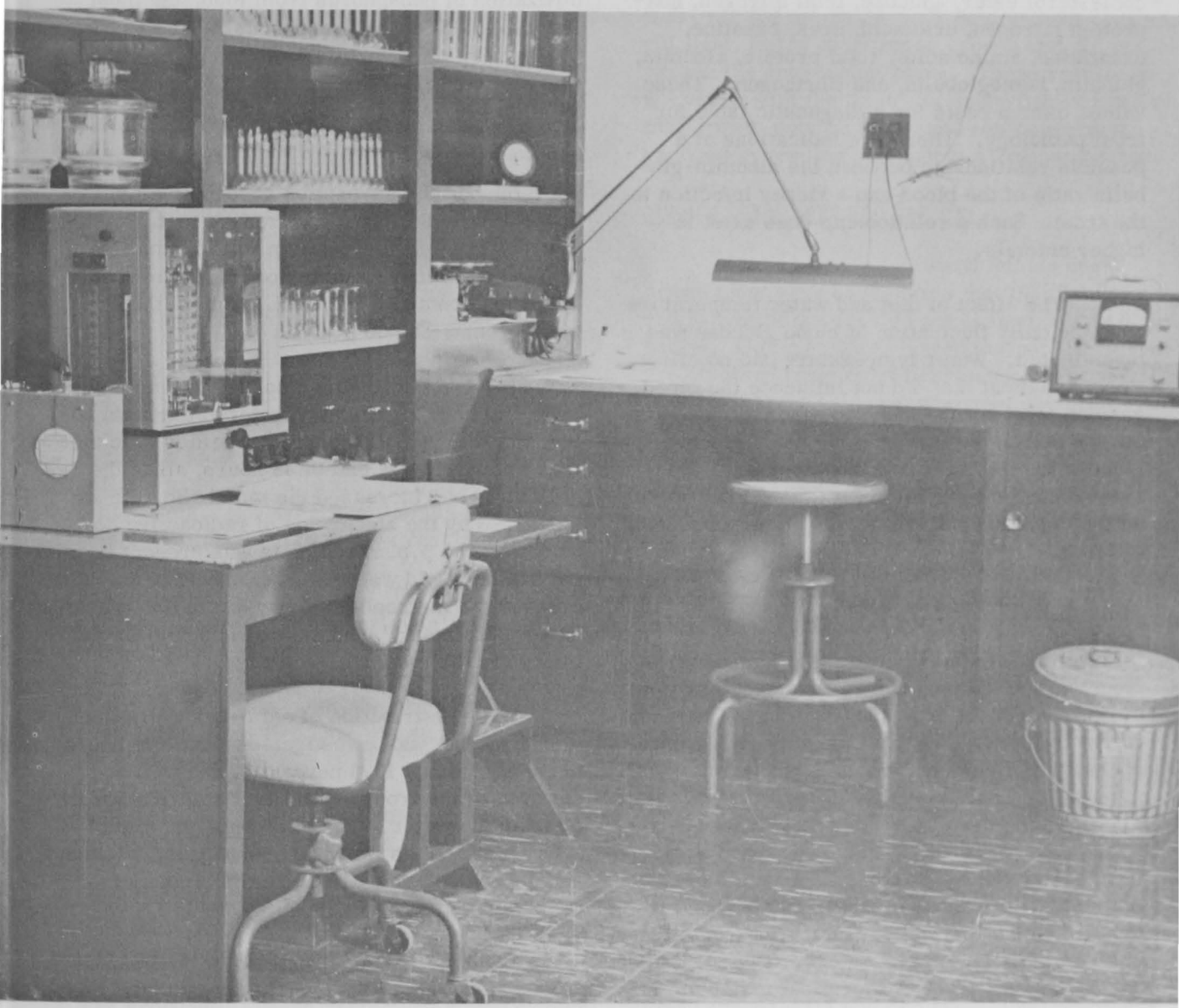
3. Mineral metabolism of trout. These studies consisted of the effect of calcium on metabolic activity, the absorption of food and water phosphorus and the factors affecting the utilization of food and water phosphorus, the effect of organic compounds and drugs on metabolic activity, and the absorption of dissolved chlorides.

4. Feeding experiments, including the evaluation of a pellet type food for brood stock, effect on growth and conversion of food calories and protein, effect of light on trout growth and conversion, and the effect of hatchery diets on body composition.

5. The physical and chemical changes of trout eggs and fry during development and incubation.

The Vitamin Requirement of Trout:

Vitamin omission studies upon lake trout established the need of these fish for riboflavin, pantothenic acid, biotin, pyridoxine, folic acid, B_{12}, inositol, choline, and niacin. Similar studies upon Atlantic salmon showed that these fish require pantothenic acid, pyridoxine, and riboflavin. Other members of the B complex were not found essential for Atlantic salmon, but the results were so close to statistical significance that the additional vitamins probably must be considered essential until further studies confirm present results. A lack of precision and the size of the experimental animal may have reduced the sensativity of these studies. Pyridoxine, riboflavin, and choline were found essential for rainbow trout. These vitamins were not reported in previous studies upon this species of fish. Both brook and brown trout exhibited the typical



South end of the radio-active tracer laboratory of the Cortland Experimental Hatchery showing the weighing and dissecting areas. This laboratory utilizes the techniques of radio-active isotopes in studying the metabolism of minerals by trout.

eye opaqueness associated with a riboflavin deficiency in trout and salmon.

The Chemical Composition of Trout Blood

Values for brook and brown trout blood were established for lactic acid, cholesterol, cholesterol ester, glucose, total nitrogen, non-protein nitrogen, uric acid, urea, creatine, creatinine, amino acids, total protein, albumin, globulin, hemoglobulin, and fibrinogen. These values offer a basis for a diagnostic table for trout pathology. There are indications of a possible relationship between the albumin-globulin ratio of the blood and a kidney infection in the trout. Such a relationship does exist in higher animals.

The effect of diet and water temperature upon the daily fluctuation of blood glucose was investigated. Water temperature had no effect. Feeding a meat diet did not influence the blood glucose level but the addition of a dry meal containing starch did increase the blood glucose level of the fish over a 12-hour period. These results can be correlated with the earlier work on the digestion and absorption of carbohydrates by trout.

The Absorption of Minerals by Trout

Studies upon the mineral metabolism of trout were made possible several years ago by the establishment of a laboratory to utilize radioactive isotopes. The laboratory is at present operated by Henry A. Podoliak, Chemist.

Using the absorption of radio active cobalt as a measure, it was found that the metabolic activity of brook trout increased more than the metabolic activity of either rainbow or brown trout when the fish were transferred from high-calcium to calcium-free waters. Two strains of hatchery brook trout had identical metabolic increases and brown and rainbow trout showed similar activity changes. These studies further emphasize the possibility of metabolic shock in stocking hatchery fish in natural waters.

Using radioactive phosphorus, it was found that brook trout absorbed only about 1/400th as much dissolved phosphorus as dissolved calcium from the water. Small structural use was made of the dissolved phosphorus. The rate of absorption was directly proportional to the amount of phosphorus in the water, except when calcium fertilization was used. Calcium fertilization increased the quantity of phosphorus absorbed. The site of absorption of dissolved phosphorus was the gills. In studies on the utilization of phosphorus from food, the most efficient calcium-to-phosphorus ratio was 1:1. The pattern of metabolism of food phosphorus was similar to the metabolism of food calcium found in previously reported experiments.

The phosphorus content of the water did not influence the absorption of food calcium by brook trout. Because of increased metabolism of brook trout in low calcium waters, the fish utilized significantly more food calcium when the water contained 5 p.p.m. calcium than when it contained 50 p.p.m.

Copper sulphate, glucose, sodium chloride, and urea significantly altered the metabolism and survival of brook trout held in calcium-free water. By the end of 48 hours, all of the chemicals had increased the metabolic rate as measured by the absorption of radioactive cobalt, when 50 p.p.m. calcium was present in the water. Cold water and prior starvation decreased the metabolic activity of the fish whereas MS 222, methyl pentynol, and sodium amytal increased the metabolic activity.

The distribution of chlorides to tissues of brook trout was investigated. After 96 hours in the aquarium, 10.8 percent of the total body chloride of the trout was labeled chloride absorbed from the water. The gills were the site of absorption. Thus we know phosphorus, calcium, cobalt, and chloride are absorbed directly from the water by trout.

Studies on the absorption of phosphorus from water showed the pyloric caeca as the main site of deposition of phosphorus. The amount of phosphorus absorbed was directly proportional to the amount available in the water. Mortalities of the fish were again high in the zero calcium water but the fish survived in water containing 0.5 p.p.m.calcium. Low water calcium increased the absorption of phosphorus to about the same extent as a 30 F. increase in water temperature. Both of these increases in phos-

phorus absorption are indicative of increases in metabolic rate of the fish.

Studies upon the utilization of phosphorus for four days after one feeding showed that maintaining a constant Ca:P ratio and increasing the level of phosphorus by a factor of four caused a proportional utilization of phosphorus from food. Water temperature had little significant effect upon the total deposition of phosphorus after four days. Increasing the calcium content of the diet increased the chemical binding of the phosphorus within the digestive tract, and thus an increased loss of phosphorus through intestinal elimination. The calcium and phosphorus content of the water influenced the utilization of phosphorus from the food. Dietary fat and carbohydrate increased the utilization of food phosphorus but trace minerals had no effect.

Feeding Trials

Brown trout, maintained over a three-year period on a diet composed entirely of Cortland pellets, grew normally and showed low daily mortality rates. However, the fish did not handle well and a loss of fish always resulted from such routine handling as weighing. These fish were spawned in the fall of 1958. The resulting reproductive products (approximately 170, 000 eggs) were of poor quality and a high egg mortality resulted. It is estimated that less than 10 percent of the eggs have reached the eyed stage at this time. The adult fish have shown a high mortality since spawning.

Values were calculated for the estimation of calories in trout diets. Based upon digestibility, the calorie values assigned were 1.6 calories per gram of carbohydrate, 3.8 of protein, and 8.0 of fat. Using these values and data from past experiments, 2, 100 calories and 350 grams of protein are required for each pound of fish produced. These values apply only to high calorie diets which were defined as those diets containing in excess of 700 calories per pound of food.

Experiments showed that by regulating the calorie and protein intake of fish, it is possible to reduce both the protein and total calories in trout diets without harming either growth or conversion rates.

Growth experiments showed that continual light over a 24-hour period, accompanied by increased food allowances, decreased the growth rate and increased the conversion in comparison to trout held under normal conditions. Feeding fish over the entire 24-hour day, without continual light, failed to increase growth and did increase the conversion rates. These results do not agree with the theory of many fish-culturists that growth may be increased and conversions reduced by feeding throughout a 24-hour period.

Studies Upon Eggs and Sac Fry

Studies by Mr. Richard Dumas upon brown trout eggs have shown that the diameter and weight of the eggs do not change during incubation. Other studies showed that smaller eggs are obtained from trout held under light control than from trout held under normal conditions and smaller eggs result from feeding a pellet-meat mixture to fish than from feeding standard hatchery diets. The eggs were larger from second spawners than from first spawners.

Studies upon the chemistry of developing trout eggs and sac fry were continued. The results were similar to those previously reported for the period of the eyed stage to sac fry. The continuance of these studies through the sac fry stage to feeding showed that the water content increased, and the fat and protein content decreased. The amount of ash remained constant during this period.

Within-Service Training School

The revision of the outline used in the training school was completed.

Five men from the Bureau of Sport Fisheries and Wildlife and two state men (representatives of New York and Virginia) are at present in attendance at the training school.

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eye opaqueness associated with a riboflavin deficiency in trout and salmon.

The Chemical Composition of Trout Blood

Values for brook and brown trout blood were established for lactic acid, cholesterol, cholesterol ester, glucose, total nitrogen, non-protein nitrogen, uric acid, urea, creatine, creatinine, amino acids, total protein, albumin, globulin, hemoglobulin, and fibrinogen. These values offer a basis for a diagnostic table for trout pathology. There are indications of a possible relationship between the albumin-globulin ratio of the blood and a kidney infection in the trout. Such a relationship does exist in higher animals.

The effect of diet and water temperature upon the daily fluctuation of blood glucose was investigated. Water temperature had no effect. Feeding a meat diet did not influence the blood glucose level but the addition of a dry meal containing starch did increase the blood glucose level of the fish over a 12-hour period. These results can be correlated with the earlier work on the digestion and absorption of carbohydrates by trout.

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Personnel

Mr. Harold Doppel reported in November 1958, to assume the duties of the second man in the isotope laboratory. Mr. Doppel is trained in biochemistry.

Mr. Donald Livingston reported in March, 1958, to assume the duties of Fish-Culturist in charge of the experimental hatcheries.

Mr. Richard Dumas reported in July, 1958, as the New York State replacement for Mr. Donald Brockway who died last January. Mr. Dumas will be concerned with biological and analytical studies in the standard laboratory.

Western Fish Nutrition Laboratory
Willard Laboratory
Cook, Washington
John E. Halver, Chief

Major emphasis of the laboratory has been directed toward accumulating definitive requirements of salmon for proteins together with complementary studies to understand the complex interrelationships between amino acids, sugars, and fats. Techniques of histology have been explored to furnish sub-clinical deficiency syndrome detection. Protein requirements were determined using whole egg protein or an equivalent amino acid mixture at various water temperatures. A direct dependence of the protein intake on water temperature was established. Nitrogen supplement compounds for the protein component of the diet were investigated and arginine found to be most effective. Using this information, specific amino acid requirements for chinook salmon were determined for histidine, methionine, threonine, and lysine. Tentative requirements were determined for arginine and tryptophan. The complete vitamin test diet was altered and improved to furnish adequate protein intake to fish in different water temperatures. The independence of the amino acid requirement on water temperature at a constant protein intake was observed. Techniques for rapid amino acid assay of diet and tissue components were investigated and adapted for practical use. The problem of D-amino acid oxidase deficiency under certain conditions was investigated with inconclusive results. Utilization of certain D forms of the amino acid for protein test conditions was determined, but the problem of which D-amino acid oxidase limits the use of racemic mixtures in salmon diets remains to be investigated.

Some water-soluble vitamin requirements for sockeye salmon were determined.

An absolute requirement of chinook salmon for fat and possibly certain unsaturated fatty acids was investigated together with studies on the protein calorie ratio of test and production diets.

The mineral test diet was improved to allow investigation of certain minerals and salt mixtures.

An interesting relationship between the water content and utilization of certain dry diets was determined.

Multiple hemoglobin types were found in various species of fish.

Electrophoretic patterns of fish sera were studied and chemotherapeutic agents for the control of Hexamita salmonis were surveyed and a number found promising.

An intense investigation of X-ray spectroscopy of urea and thiourea adducts of fats resulted in fundamental contributions to the stereo configuration of fatty acids and fish lipids.

Histological surveys were run on thiamin, pyridoxine, pantothenic acid and riboflavin deficient fish and in certain cases were compared with induced vitamin deficiencies in rat tissue.

Techniques were improved to select more descriptive materials during an experiment. Hyperthyroidism was successfully induced by feeding iodocasein to salmon.

Niacin-deficiency was closely correlated with types of sunburn.

Dicumarol produced anemia and mild hemorrhage but gross lesions of vitamin K deficiency were not observed.

Hematology of salmonids was initiated.

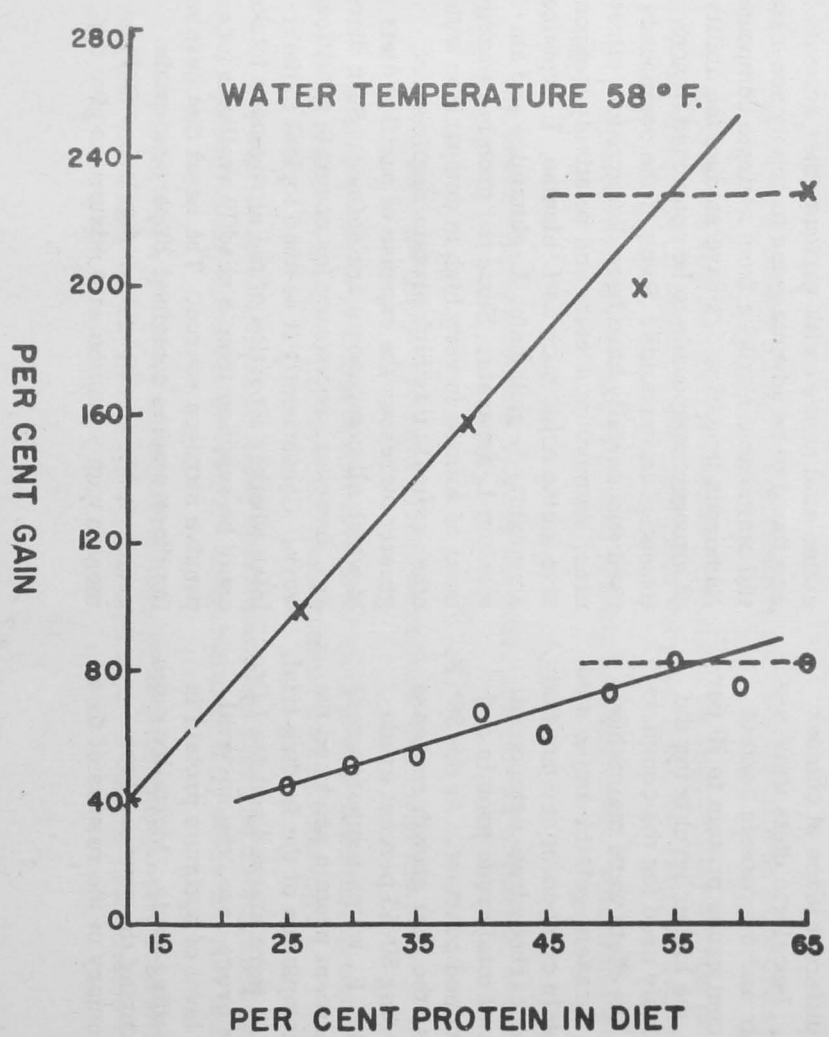
NUTRITION

Protein requirements

Knowing that 10 amino acids are required for growth and maintenance by chinook salmon, the gross protein requirements were investigated as a logical precursor for determining what protein levels should be fed during an amino acid quantitation study. Whole egg protein was selected as the best protein available because of its high indispensable amino acid content and because in every other experimental animal studied, whole egg protein had the highest biological value. Using chinook salmon, these assumptions were validated and an interesting relationship developed between gross protein intake and water temperature. Two series of experiments were completed at 58° F. and 46° F. water temperatures with different sizes of chinook salmon fingerlings. Isocaloric diets were used in these experiments and the protein content varied from 25 percent crude protein to 70 percent crude protein, the higher level being the same as that previously used for the complete vitamin test diet. The diets were maintained isocaloric by using a casein-gelatin-amino acid mixture for the protein component and purified white dextrin for the carbohydrate component, with the summation of total crude protein and carbohydrate maintained constant. At the 58° F. water temperatures, the best growth response was obtained by feeding 50-55 percent crude protein and at the 46° F. water temperatures, approximately 40 percent protein produced the best gain. At the completion of the feeding trial, individual lots of fish were starved and the LD_{50} time calculated. In every case, the survival data confirmed the level of optimum protein intake found by the feeding study. Mortality rates were insignificant during the course of the experiment. A summary of the results of these studies can be visualized in figure 1. The diets were fed ad libitum on a rigid schedule three times daily, six days weekly with no food offered on Sundays. A complete description of the experiments with full experimental details and a discussion of the results has been published (DeLong, et al 1958). It would be interesting to calculate the formula of the curve connecting these two water temperature points by determining one or more intermediate points because one could then calculate the optimum protein requirement (providing the indispensable amino acid content of the protein fed was satisfactory) for any water temperature harboring experimental or production hatchery salmon.

Nitrogen supplements for salmon diet studies

In the development of purified diets for amino acid studies with various other animals it was found to be advantageous to supply non-essential amino acid nitrogen from a single compound. Numerous investigators have studied the ability of nitrogen compounds to be converted to non-essential amino acids. Some of the compounds used successfully have been diammonium citrate urea, ammonium acetate and relatively inexpensive amino acids such as L-alanine, L-arginine, ammonium L-glutamate, L-glutamine and ammonium L-asparate. Since the protein requirement of salmon is very high in comparison with other animals this high protein requirement greatly increases the expense of purified diets in which all components are defined. Such diets are, however, necessary for accurate nutritional work. Consequently it seemed logical to determine whether a portion of the nitrogenous intake could be supplied from a readily available inexpensive nitrogen source. The basal diet developed for these studies contained 20 percent crude protein made up of casein and gelatin supplemented with an amino acid mixture to give the



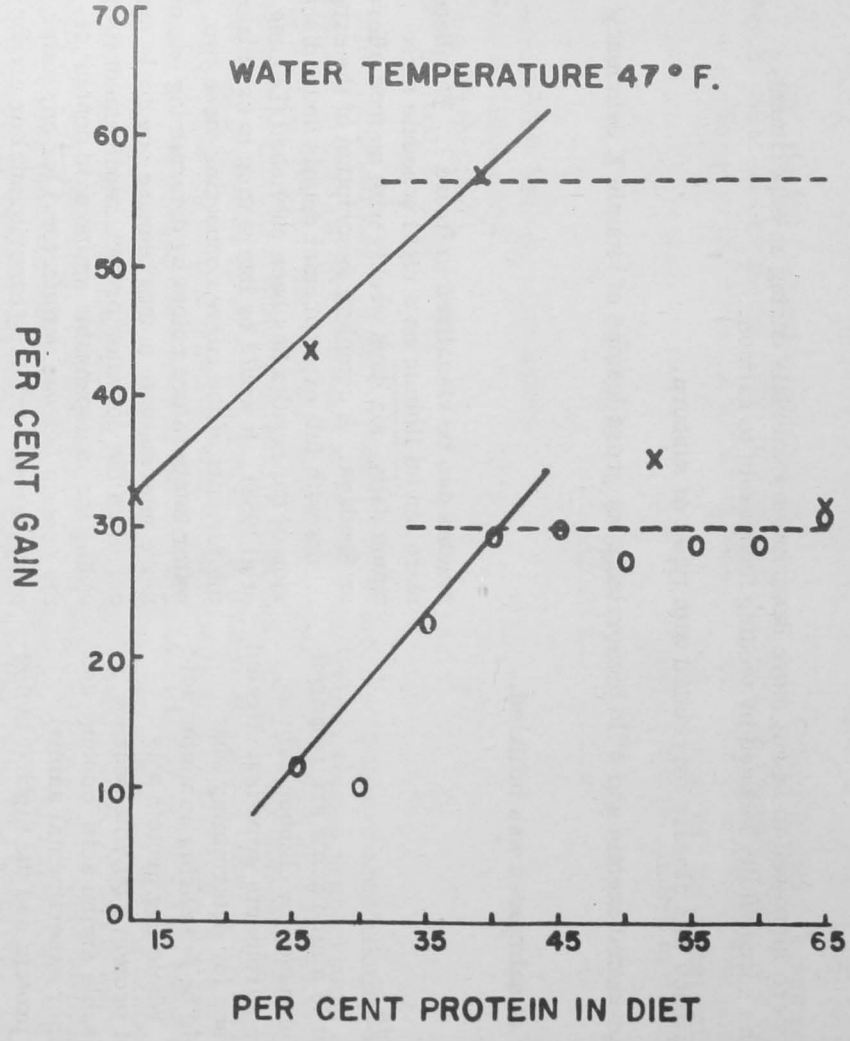


Figure 1. Growth response of chinook salmon fed different protein increments for 16 weeks. The slopes of the sets of curves vary because of the different sizes of fish used at the start of the experiment.

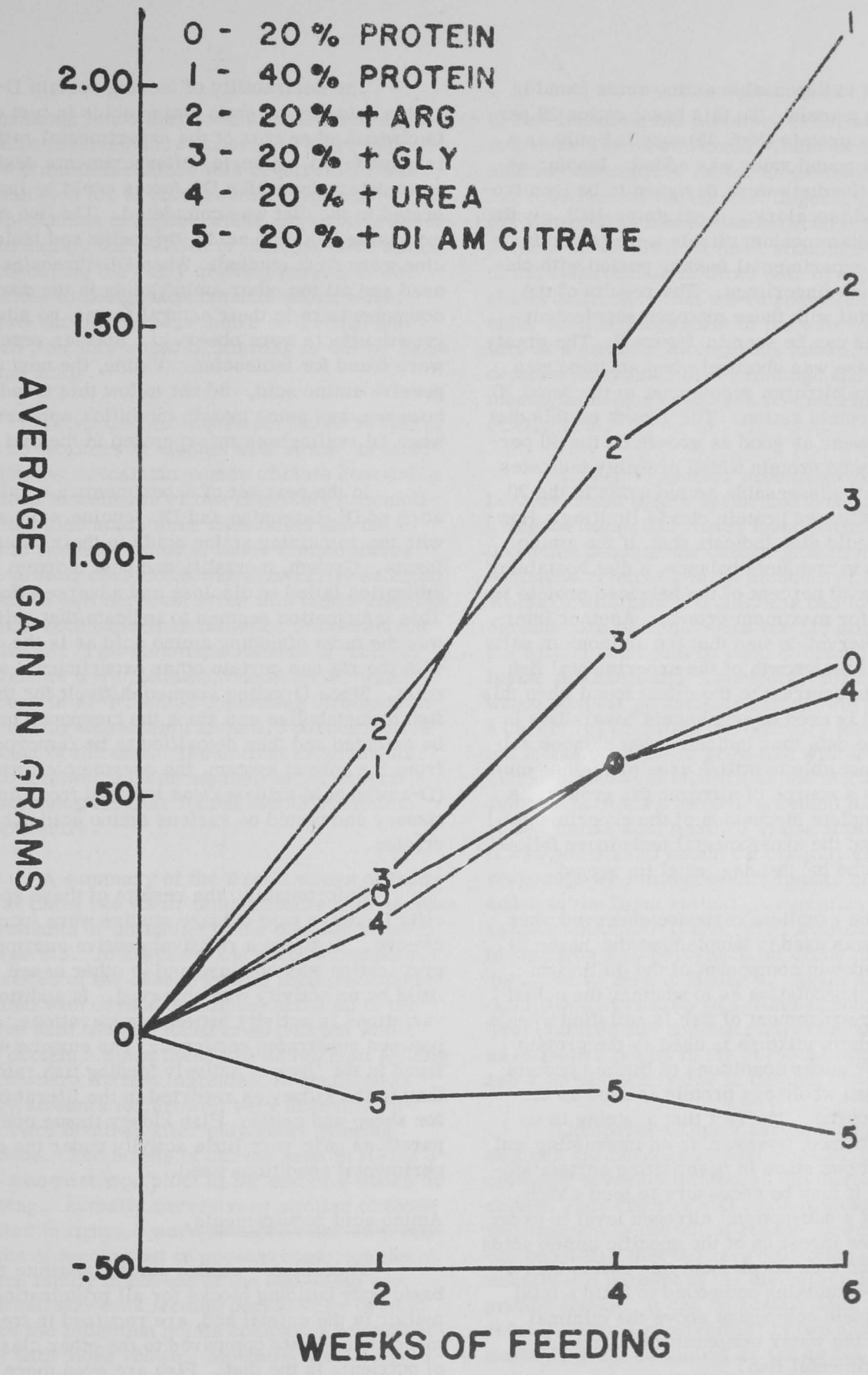


Figure 2. Growth of experimental fish fed diets containing various sources of nitrogen for the protein component.

balance of indispensable amino acids found in whole egg protein. To this basal ration 20 percent crude protein (Nx6.25) supplied only as a single compound study was added. Insofar as possible, the diets were designed to be isonitrogenous and isocaloric. L-arginine-HCl, glycine, urea and diammonium citrate were tested for a six-week experimental feeding period with chinook salmon fingerlings. The results of the feeding trial with these nitrogen supplement compounds can be seen in figure 2. The greatest response was obtained when arginine was used as the nitrogen supplement to the basal 20 percent protein ration. The growth on this diet did not appear as good as growth on the 40 percent balanced protein which probably indicates one of the indispensable amino acids in the 20 percent balanced protein diet is limiting. However, it could also indicate that, if the amino acids are in complete balance, a diet containing more than 20 percent of the balanced protein is required for maximum growth. Another interesting observation was that the ammonium salts depressed the growth of the experimental fish which is in contrast to the effect found when this compound is used to supplement basal diets in rats. The data thus indicates that chinook salmon are not able to utilize urea or diammonium citrate as a source of nitrogen for growth. A more complete discussion of the experimental results and the experimental techniques followed can be found in DeLong, et al (in press).

The excellent response observed when arginine was used to supplement the basic 20 percent protein component of the diet might stimulate speculation as to whether the actual arginine requirement of fish is satisfied when a casein gelatin mixture is used as the protein source, or under conditions of limited protein intake when whole egg protein is used as the protein source. The fact that arginine is so readily utilized, however, is an interesting and important one since in quantitating certain amino acids it may be necessary to feed a rich protein at a sub-optimal nitrogen level in order to limit the ingestion of the specific amino acids studied and then supplement this ration with a nitrogen-containing compound to build a total crude protein component above the minimal found for the water temperature surrounding the experimental fish.

The desirability of feeding certain D-amino acids or racemic amino acids in test diets is obvious when cost of the experimental ration is considered. A series of experiments designed to determine which DL forms could be incorporated in the diet was completed. The two most expensive L-amino acids, threonine and isoleucine were first studied. When DL-threonine is used and all the other amino acids in the nitrogen component are in their natural forms, no adverse growth effects were observed. Similar results were found for isoleucine. Valine, the next expensive amino acid, did not follow this trend, however, and some growth inhibition appeared when DL-valine was incorporated in the diet.

In the next set of experiments a combination of DL-threonine and DL-leucine was used with the remaining amino acids in their natural forms. Growth, mortality and post mortem examination failed to disclose any adverse effect. This information seemed to indicate that valine was the most offending amino acid as is the case with the rat and certain other experimental animals. Since D-valine seemed difficult for the fish to metabolize and since the compound must be oxidized and then detoxified to be removed from the animal system, the enzyme concerned (D-amino acid oxidase) was isolated from fish tissues and tested on various amino acid substrates.

Unfortunately, the results of these specific D-amino acid oxidase studies were inconclusive. At times a relatively active enzyme preparation was obtained and in other cases little or no activity was observed. In addition, variations in activity between preparations, and between substrates occurred. The enzyme was found in the liver of actively feeding fish rather than in the kidney as reported in the literature for sheep and cattle. Fish kidney tissue preparations gave very little activity under the experimental conditions used.

Amino acid requirements.

Threonine: -- Amino acids constitute the basic body building blocks for all proteinatious matter in the animal and, are required in tremendous amounts compared to the other classes of nutrients in the diet. Fish are even more

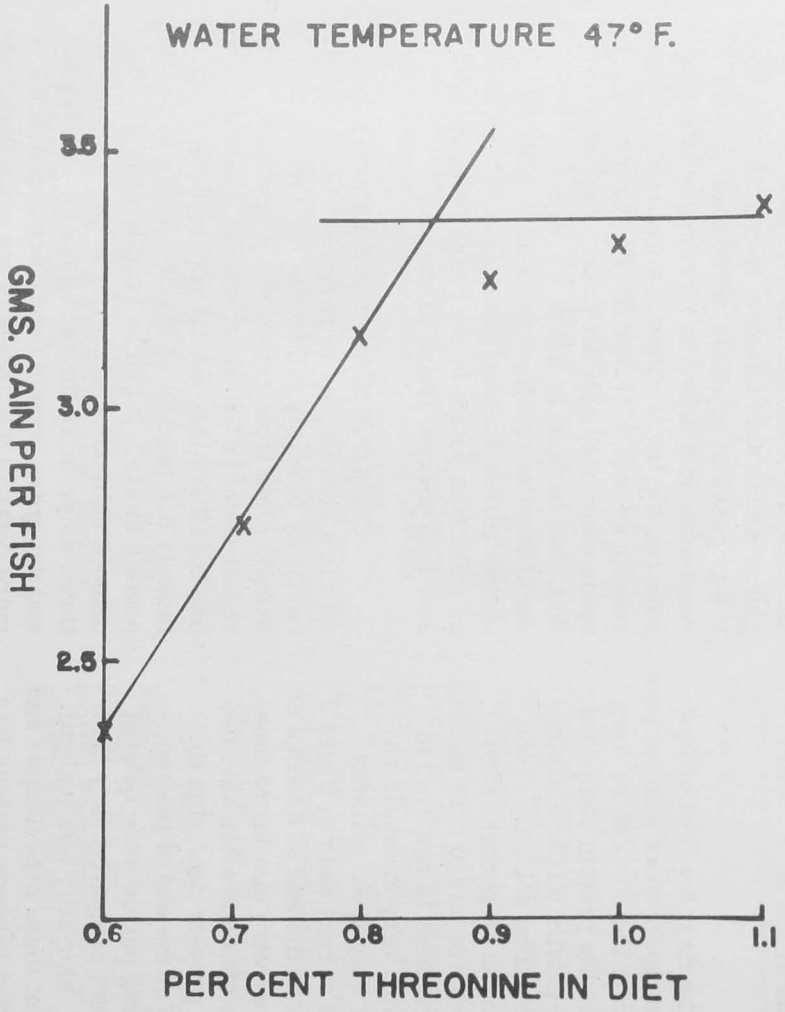
demanding, since they have an extremely high protein requirement compared to other experimental animals. Since this requirement was established for at least two experimental water temperatures and since whole egg protein seemed to satisfy the balance of indispensable amino acids during the early growing period, it was possible to design experiments which would satisfy the nitrogenous intake of the fish and which probably would be limiting in one or more amino acids.

Using the techniques perfected by Mertz and co-workers in studies with swine, an isonitrogenous, isocaloric series of diets containing various increments of two relatively uncomplicated amino acids were assembled and fed to fish at two experimental water temperatures. The protein component was arbitrarily selected at the 40 percent level since this intake could be used at both experimental water temperatures available. Two simultaneous sets of these experiments were initiated, one in 58°F. water and one in 47°F. water containing threonine at increasing increments from 0.6 percent to 1.8 percent of the diet. These first experiments were followed up with another set of experiments using smaller fish at the 58°F. water temperature.

A summary of the first 8 weeks of feeding at these two water temperatures with various increments of threonine in the diet can be seen in figure 3. In all cases the protein component consisted of the casein-gelatin mixture supplemented with crystalline amino acids up to the amino acid balance found in 40 percent whole egg protein. Since these are amino acid feeding tests where the test ingredient is required in large amounts for growth, very dramatic results are obtained after only short growing periods. The family of curves seen in figure 3 are Almquist type plots at the end of 8 weeks of feeding. Actually curves very similar to those plotted in figure 3 were obtained after only two weeks of feeding but to prevent confusion the entire family of curves at the end of the two, four and six-week feeding period were deleted since the inflection points compared very favorably with those shown. Mortality was insignificant during the course of these experiments and the total food consumption between various lots of fish including the extremes in threonine content were almost constant. Therefore, we were able to demonstrate rather elegantly that threonine requirement for chinook fingerlings with 40 percent protein intake lies between 0.8 and 0.9 percent of the diet. A most striking duplication of the requirement in three sets of experiments shows concretely that the requirement for threonine seems independent of the water temperature at a constant nitrogenous intake. A more detailed discussion of the findings and experimental techniques used can be found in Halver, et al., 1958.

Lysine:--Another protein source had to be considered before lysine could be quantitated using the same techniques found satisfactory for threonine because the casein-gelatin mixture contained relatively large amounts of lysine. Workers with rats and chickens had found that certain corn proteins were readily utilized by the animal and could be used to determine the lysine requirement. Therefore, corn gluten was tested for palatability and growth and after a carefully controlled introductory feeding period it was possible to grow fish with a corn gluten-amino acid mixture as the nitrogenous component. After the fish accepted the corn gluten-amino acid mixture-lysine limiting diet, it was possible to obtain differential growth responses with different increments of lysine added to the basal ration. A summary of these results at both 47°F. and 58°F. water temperatures using a 40 percent basal crude protein diet, can be seen in figure 4. Again a close duplication of results was found at both water temperatures and duplicate experiments yielded inflection points in the curve between 2.05 and 2.35 percent lysine in the diet.

Compared to other experimental animals this is a tremendous requirement for lysine and certainly severely influences the formulation of salmon diets from cereal grain proteins. This requirement is not out of reason, however, when one considers that the rat requirement is approximately 0.8 percent of the diet with a 16 percent protein intake. It can be readily seen that the chinook salmon requirement is two and one-half times as great as the rat as far as both lysine and the gross protein intake are concerned. A more detailed discussion of the experiment can



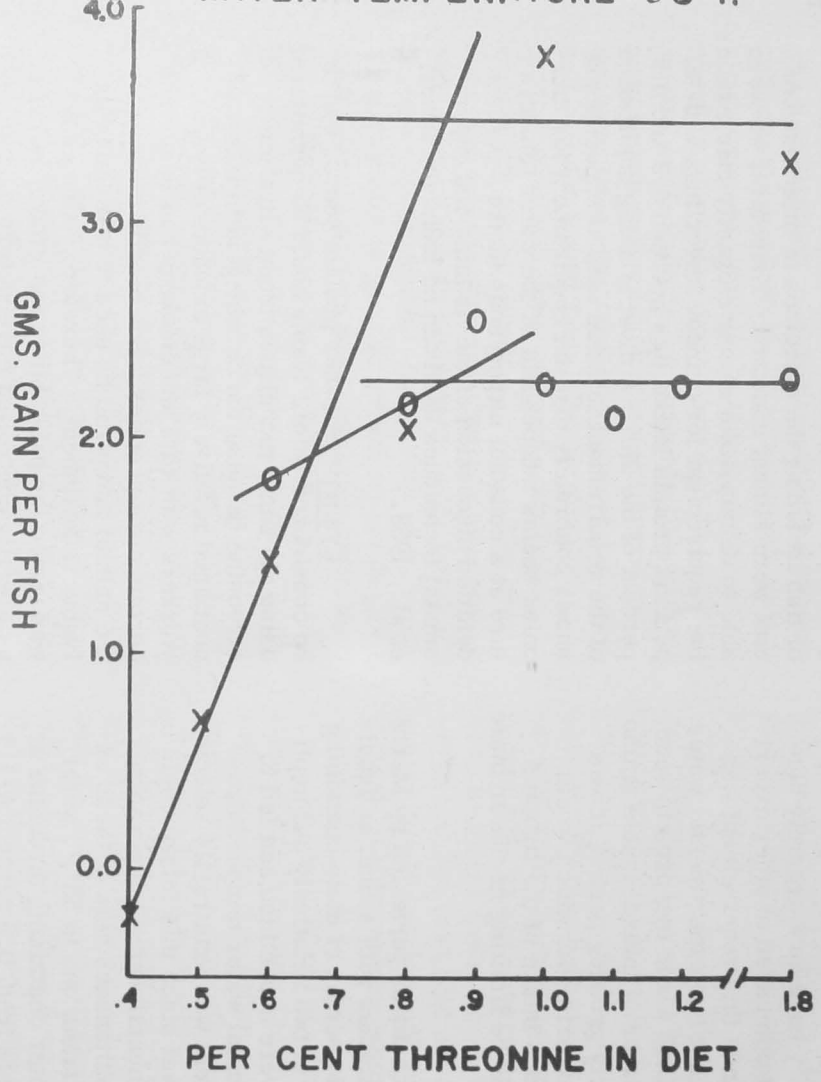


Figure 3. The threonine requirement of chinook salmon.
These are summary plots at the end of 8 weeks of feeding paired lots of chinook salmon fingerlings a 40% protein diet containing the amounts of threonine specified. The junction points of the straight lines drawn represent the threonine requirement of the fish under the experimental conditions used. Differences in the slope of the growth line occur due to differences in size of the experimental fish at the start of the feeding trial.

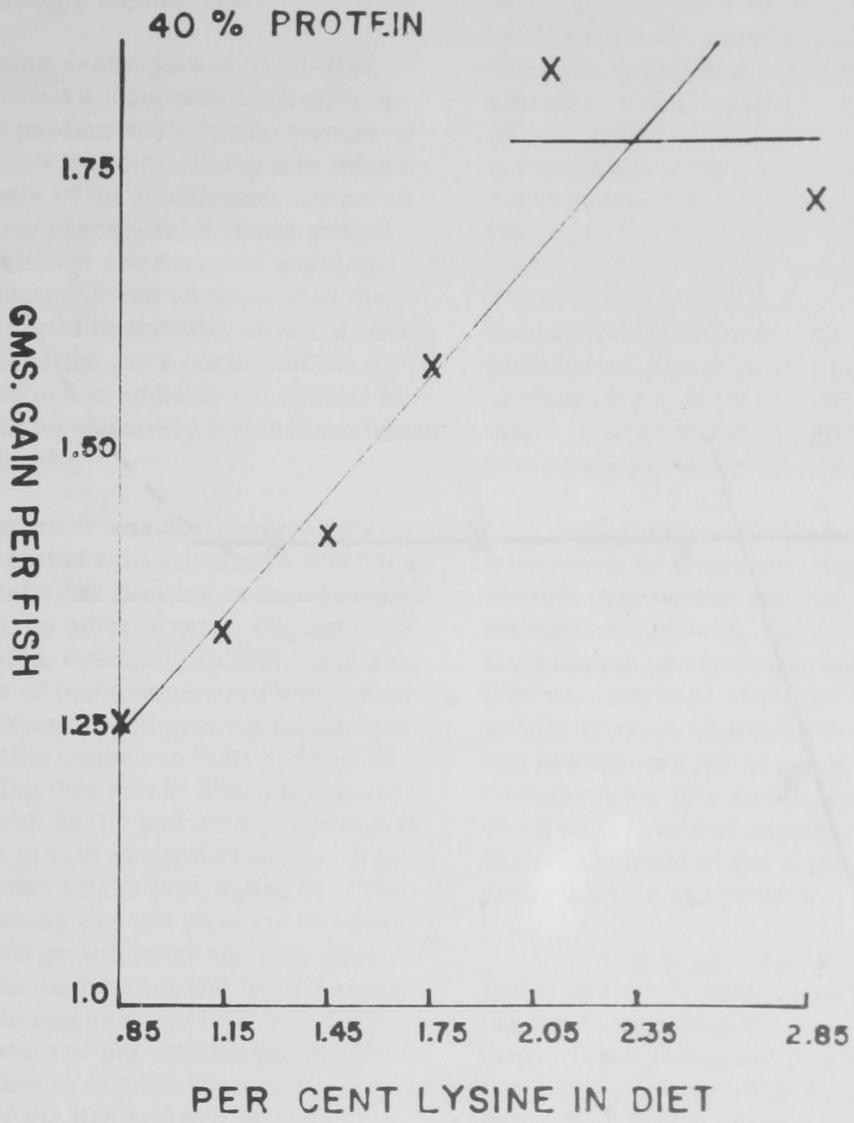


Figure 4. Lysine requirement of chinook salmon.
This is a composite plot of two sets of experiments at two water temperatures, 47° F. and 58° F. The slopes of the two families of curves were fortuitously identical because the experiment in 58° F. water was started later with larger fish tending to compensate for the more rapid growth in the warmer water.

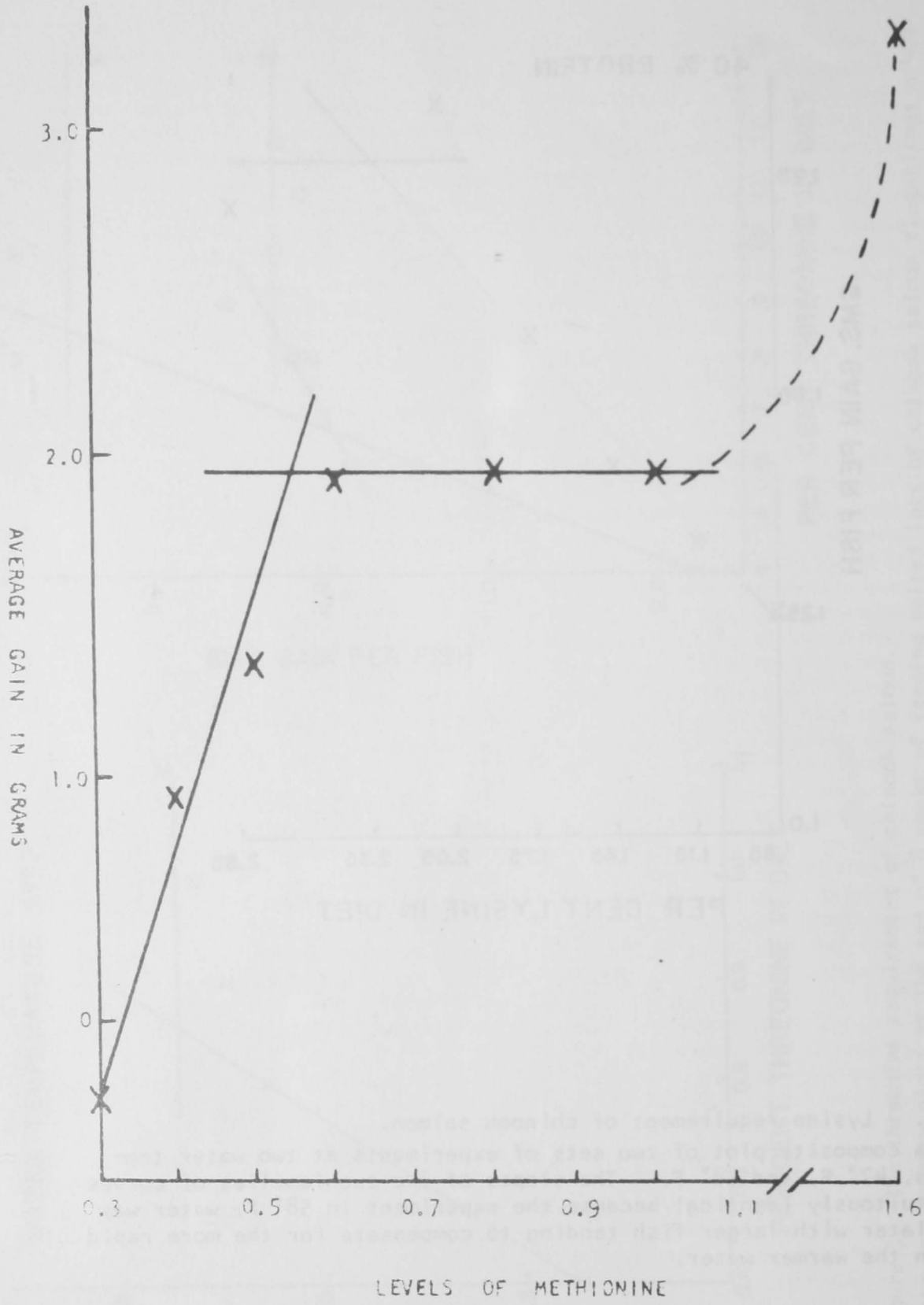


Figure 5. Methionine requirement without cystine.

The first six points plotted indicate the growth responses obtained when cystine is held constant at 0.05% of the diet. Note the tremendous response when 1% cystine is added and the methionine content is the same as in 40% whole egg protein (last point).

be found in DeLong's thesis, 1958.

Methionine:--Analysis of whole fish carcasses indicated a threonine content of approximately .9 percent and a lysine content of approximately 2.0 percent. Using this information and analysis of the methionine content of salmon fingerling carcasses, a tissue projection of the methionine requirement would fall somewhere between .5 and .6 percent of the diet. Hence, it would be possible to use a casein-gelatin combination for a portion of the nitrogenous intake and in addition the cystine requirement would be relatively low if these whole proteins were used.

An experiment was designed utilizing a casein-gelatin-amino acid mixture to furnish a 40 percent protein diet limiting in methionine and very low in the other organic sulphur containing amino acid, cystine. To this basal ration increments of methionine were added over the range .3 percent to 1.6 percent methionine holding the cystine content to 0.05 percent of the diet. Feeding this diet to chinook salmon fingerlings at both 58°F. and 47°F. resulted in a plateau in the growth at approximately .6 to .7 percent methionine intake (see figure 5). The slope of this plateau was not constant however, for a tremendous growth response was obtained when 1.6 percent methionine, the level found in 40 percent whole egg protein, was fed. This indicated that some of the methionine was probably being utilized to furnish the organic sulphur requirements of the fish and that methionine might not be able to be metabolized rapidly enough to satisfy the cystine demand. It should be possible to prove this hypothesis if the total organic sulphur content of the diet were increased above that point demanded by the fish for rapid growth. Consequently, a second series of experiments were designed in which the cystine content was held constant at approximately 1 percent of the diet. In these experiments a sharp break in the Almquist type plot occurred and the methionine requirement was indicated at approximately 0.5 percent of the diet. Further details of the experiment can be found in Halver, et al (in press).

Histidine:--Determination of the histidine requirement of chinook salmon was made possible by using a casein-gelatin-amino acid mixture for the protein component limiting in histidine and adding various increments of histidine to the ration to establish the range desired. Little difficulty was encountered in these experiments and in two sets of runs with two sizes of chinook salmon fingerlings, sharp deflections in the Almquist type plots were obtained between 0.4 and 0.5 percent of the diet. These values compare very favorably with tissue projection analysis and the prediction of the histidine requirement at approximately 0.4-0.5 percent of the diet with a 40 percent protein intake. A more complete discussion of these experiments can be found in DeLong's thesis, 1958.

Arginine:--The observation that tremendous amounts of arginine may be utilized to furnish some of the non-essential amino acid nitrogen for growing salmon fingerlings raised the question of whether adequate arginine intake was satisfied when the standard casein-gelatin mixture or when even 40 percent whole egg protein was fed to young salmon. The requirement for this amino acid will not be elucidated until levels of arginine fed are extended beyond the point of the arginine content in 40 percent whole egg protein.

Tryptophan:--Knowing the threonine, lysine and methionine requirements and analyzing the salmon fingerling carcasses for tryptophan, tissue projection predicted the tryptophan requirement at approximately .19 percent of the diet. With the techniques previously used, tryptophan would be difficult to quantitate even if only one-third of the nitrogenous intake consisted of the casein-gelatin mixture. Our work on tryptophan will be repeated using a different source for the native protein component which would limit the tryptophan intake below approximately .1 percent dietary intake. This effect can be accomplished either by selecting other proteins or feeding a lower percentage of native protein and increasing the amino acid mixture content of the diet.

Water-soluble vitamin requirements

Silver salmon:--The indispensable water-soluble vitamins were known for chinook salmon, together with the specific deficiency syndromes

induced when one vitamin is missing from the diet. Only chinook salmon fingerlings had been thoroughly studied and it appeared desirable to test the same experimental techniques with silver salmon, an important species hatchery-reared in large numbers by the State of Washington Department of Fisheries and certain Federal hatcheries. Since this project was of mutual interest to both the Federal and State agencies, a cooperative research program was started with the State of Washington Department of Fisheries to test the qualitative vitamin requirements of silver salmon. The State provided a portion of the Issaquah Hatchery located at the head of Lake Sammamish near Seattle, Washington, together with the necessary fish troughs and general supplies. They also agreed to furnish personnel to complete the experiment after it was initiated by members of the laboratory group. Our laboratory furnished dietary ingredients and assigned Mr. Duane Gahimer to the Issaquah station for a period of 6 weeks to initiate the vitamin feeding studies. He carried the salmon fingerlings through the preparative stage, set up the individual troughs, mixed the first set of diets and vitamin mixtures, and demonstrated to the State personnel the techniques used by the laboratory for conducting vitamin deficiency feeding studies. Mr. Daniel C. Coyle, an Issaquah High School science instructor was hired by the State to carry on the experiments and the Washington Department of Fisheries assigned Mr. Jack Coates, their nutritionist, to supervise the details of the project. The State also furnished the services of Mr. Brian Earp to study the appearance of any fish disease during the course of the experiment and to help detect specific vitamin deficiency syndromes as they occurred in various lots of fish.

The design of the experiment was almost identical to the one used for determining the water-soluble vitamin requirements for chinook salmon fingerlings and was designed for an 18-week feeding period in case some of the deficiency syndromes appeared late in the feeding trial due to inadvertent introduction of natural food particles from the hatchery water supply.

After the appearance of specific vitamin deficiency syndromes in the majority of the individual population of fish and after a loss of approximately 20 percent of the individual population, that particular trough was divided in half, one-half of the survivors was continued on the deficient ration until the entire population was lost and the lower one-half of the trough was fed the complete vitamin test diet. The recovery of the individual fish from the specific deficiency syndrome was carefully observed.

Periodic samples for chemical and histological analysis were collected during the course of the experiment and were shipped to the laboratory at the termination of the feeding trials for subsequent analysis by our group. A low level incidence of Hexamita salmonis appeared in most lots of fish on the Issaquah Creek water supply, but no serious epizootic appeared and the majority of the specific vitamin deficiency syndromes could be detected and classified for silver salmon. A typical set of growth curves can be seen in figure 6.

Under these experimental conditions it was possible to describe specific vitamin deficiency syndromes in silver salmon for thiamin, pyridoxine, folic acid, biotin, pantothenic acid, inositol and choline. Inconclusive results were obtained with the nicotinic acid, vitamin B_{12} and riboflavin deficient lots of fish. No abnormal indications in growth, appetite or mortality were observed for ascorbic acid or paraminobenzoic acid deficient groups. The complete list of specific silver salmon water-soluble vitamin deficiency syndromes compared to those observed by other workers in other fish can be found in Coates and Halver, in press.

Sockeye salmon:--The possibility of using the complete vitamin test diet of chinook salmon for other species of salmon was enhanced by the finding that silver salmon seem to grow well on this diet and specific vitamin deficiency syndromes could be induced in individual lots by deleting one of the crystalline water-soluble vitamins from the vitamin supplement. Space was at a premium in our experimental hatchery but in the aquarium room there were twelve ten-gallon aquariums equipped with circulating water which could be used for feeding small lots of young salmon. Therefore, as a training exercise for Mr. James Bayman, a fish culturist from the Willard Hatchery on temporary duty

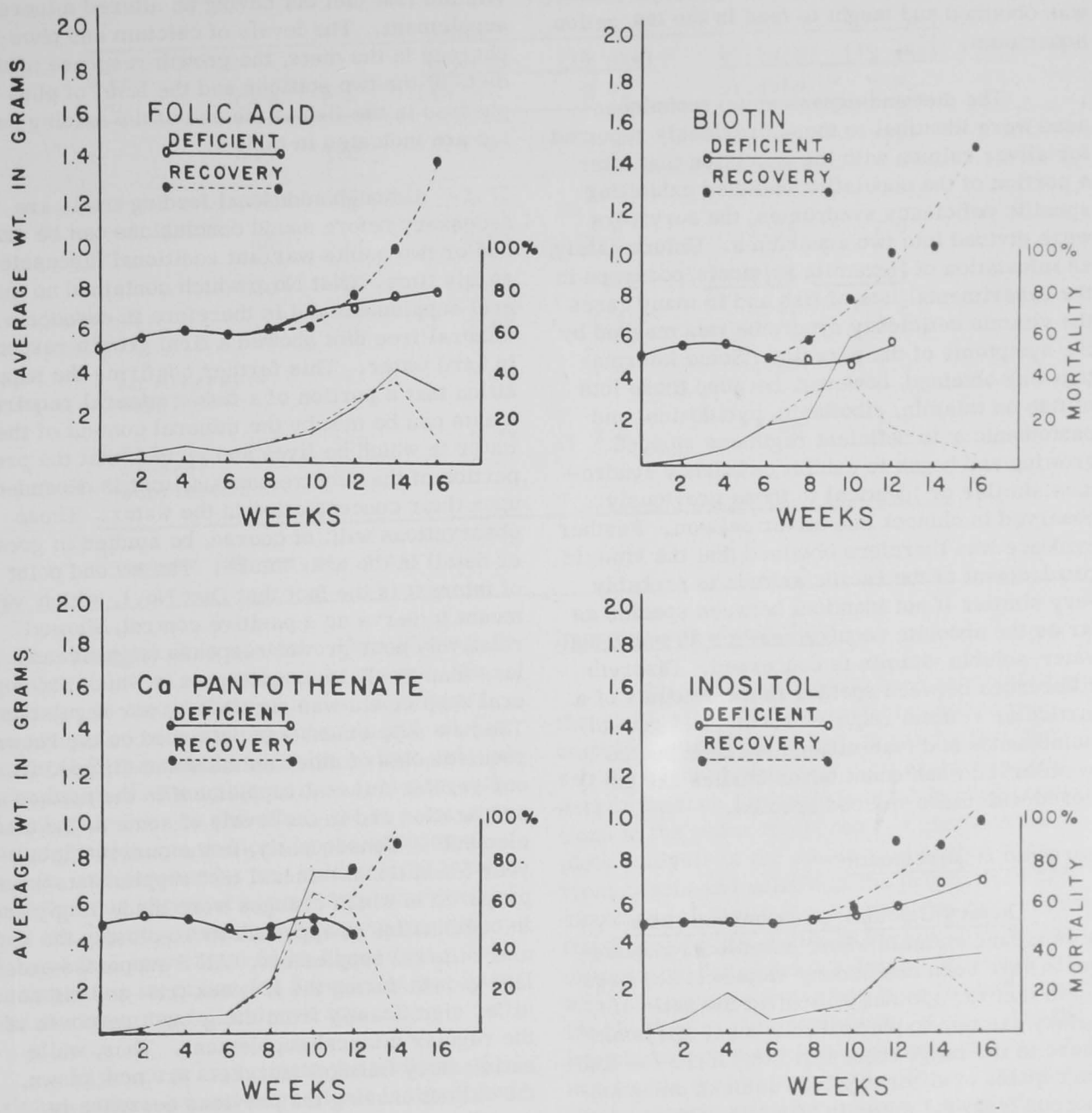


Figure 6. Typical growth curves of vitamin deficient silver salmon. The junction points in the growth and mortality curves reflect the division of the original population into two equal sub groups. The dotted lines show the response after the missing vitamin was replaced in the diet.

at the laboratory, a small lot of sockeye salmon was obtained and taught to feed in the ten-gallon aquariums.

The diet and experimental techniques used were identical to those previously reported for silver salmon with the exception that after a portion of the population had died exhibiting specific deficiency syndromes, the survivors were divided into two aquariums. Unfortunately, an infestation of Hexamita salmonis occurred in the experimental lots of fish and in many cases the vitamin deficiency syndrome was masked by the symptoms of the parasite. Some information was obtained, however, because those lots of fish on thiamin, riboflavin, pyridoxine, and pantothenic acid deficient regimens stopped growing and began to exhibit deficiency syndromes similar or identical to those previously observed in chinook and silver salmon. Further evidence was therefore obtained that the vitamin requirement of the Pacific salmon is probably very similar if not identical between species as far as the absolute requirement for an individual water-soluble vitamin is concerned. Discrete differences between species in the amounts of a particular vitamin required for normal growth, maintenance and maturation may be expected to be observed when quantitation studies are explored with these various species.

Inorganic requirements

Dietary Calcium: phosphorous ratio:--In most land animals whose nutritional requirements have been extensively studied it has been shown that for optimal utilization the ratio of dietary calcium to phosphorous must fall somewhere in the range from 1:1.5 to 1.5:1. As fish are capable of absorbing both calcium and phosphorous from the water in which they live one would not expect this relationship to hold true. In fact, if a fish's aqueous environment contains all the inorganic elements required for life one might expect either no dietary requirements or at least that the dietary requirements would be influenced by the amount available from the environment. During the preceding fiscal year Neal Woodall set up a feeding trial both at Hagerman (hard water) and at Willard (soft water) in which both the calcium: phosphorous ratio and content were altered. The diet used was the vitamin test diet but having an altered mineral supplement. The levels of calcium and phosphorous in the diets, the growth response of the diets at the two stations and the level of phosphorous in the fish at the end of the feeding period are indicated in table 1.

Although additional feeding trials are necessary before sound conclusions can be drawn, one or two points warrant additional discussion at this time. Diet No.6 which contained no mineral supplement and is therefore in essence a mineral free diet showed a firm growth response in hard water. This further confirms the supposition that a portion of a fish's mineral requirements can be met by the mineral content of the water in which he lives and further that the proportion of the requirements so met is dependent upon their concentration in the water. These observations will, of course, be studied in greater detail in the near future. The second point of interest is the fact that Diet No.1, which was meant to serve as a positive control, showed relatively poor growth response (significantly less than No.7, its counterpart in which the mineral supplement was supplied by our regular mix). The new supplement was patterned on the known requirements of other animals and differed from our regular mineral supplement in the method of preparation and in the levels of some of the trace elements. Consequently, in the current fiscal year 5 additional mineral test supplements were prepared in which changes were made singly and in combination to approach more closely the regular mineral supplement. All 5 supported excellent growth during the 12-week trial and did not differ significantly from the growth response of the regular mineral supplement. Thus, while satisfactory mineral test diets are now known, the culprit causing the previous decrease in growth is being actively pursued.

Supplements for liver diet:--Beef liver has long been used as a control diet for tests on production diets. In view of the fact that liver protein has a relatively high biological value and that liver is an excellent source of vitamins, it seems surprising that liver usually comes out second best to good production diets. Two possible reasons for this suboptimal growth response are the mineral contents--both calcium and iodide are extremely low in liver--and the

Table 1.--Growth summary Ca:P ratio studies.

Diet No.	Ca *	P *	Hagerman (14 wks) Wt.gain	Hagerman (14 wks) % P.	Willard (19 wks) Wt.gain	Willard (19 wks) % P.
1	400	380	1.57	2.3	1.69	2.2
2	34	110	2.57	2.0	2.80	1.7
3.	40	400	2.04	2.0	2.59	2.0
4.	400	36	2.61	1.7	2.75	1.5
5	34	36	2.35	1.7	2.72	1.5
6 no mineral supplement	-	-	2.09	1.9	1.67	2.0
7 std. mineral supplement	300	350	2.73	2.0	2.48	2.2

* mg per 100 gms dry diet.

relatively high protein content. Neal Woodall designed two sets of experiments to answer this question. In one, sodium chloride (2 percent) was compared with sodium chloride and calcium citrate (1 percent each) and with sodium chloride and calcium citrates plus a trace (.01 percent) of sodium iodide. In the other, the percent protein of the diet was reduced by the addition of dextrin, and percent fat was held relatively constant by the addition of appropriate quantities of corn oil. In this manner the percent protein was reduced in 5 steps from 66 to 41 percent. Both feeding trials were run for 8 weeks at our Hagerman station using fall chinook fry.

In the first experiment the addition of calcium citrate caused a small but insignificant increase in growth while the addition of sodium iodide resulted in an approximately equal decrease. The growth results of the second experiment are presented graphically below. These results tend to confirm the data obtained with purified diets that the optimal percent protein at 58° F. water temperature lies in the vicinity of 55 percent. (see figure 7)

Improvement of test diets

Evidence has rapidly been accumulating that the levels of some nutrients used in the complete vitamin test diet, in the fat test diet and the carbohydrate test diet, are not in the proper balance for experimental feeding conditions in the water temperatures used. A complete analysis of the amino acid contribution from casein and gelatin in the proportions previously used does seem to meet the requirement for those specific amino acids known to be indispensable for chinook salmon. However, the total nitrogenous intake has been in conflict with the findings of the protein level studies and the protein:calory ratio found recently to be most acceptable for rapid growth. Therefore, as a first approximation for improvement in the test diet used for many studies in this laboratory, the proportions of casein and gelatin were reduced to a 38 parts casein-12 parts gelatin diet, with the difference being made up with white dextrin. This diet was used at the Hagerman experimental laboratory and at the Willard laboratory during the past year with favorable results, but the growth response with this new control diet still did not duplicate that of a

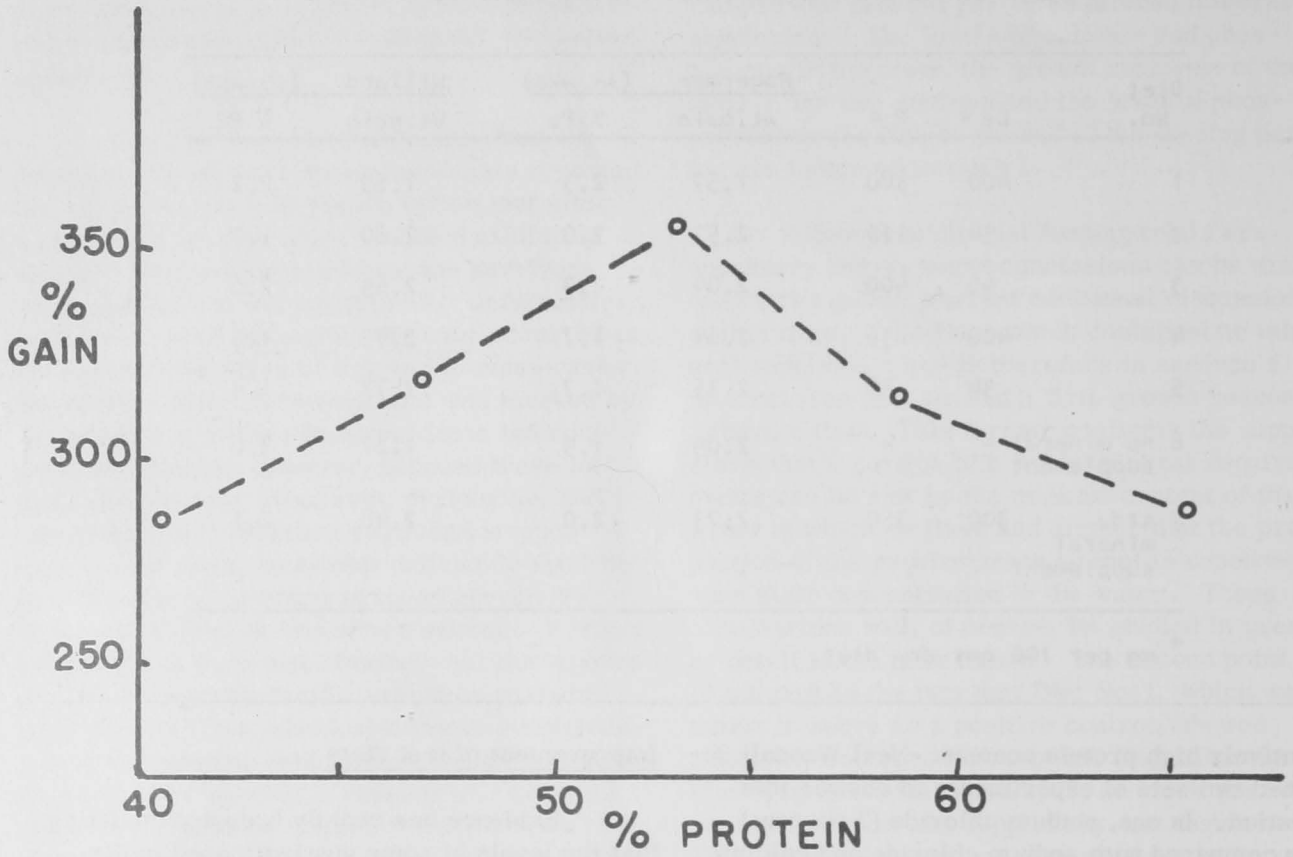


Figure 7. Growth of salmon on dextrin supplemented beef liver diets.

Each point represents the total percent gain in the 8-week feeding trial; gain is plotted versus the resultant percent protein in each diet.

liver diet to which sufficient white dextrin had been added to reduce the protein content to the order of 50-60 percent. The growth approximated that of some of the control diets used in the amino acid quantitation studies at a 40 percent whole egg protein level.

A calculation of the contribution of casein and gelatin in this proportion to the amino acid balance of the diet indicated five possibilities which are being explored. Some of the early work can be reviewed in Halver and Coates, 1957.

Adding water to dry diets:--Commercially prepared, pelleted fish foods have achieved a degree of success with trout and some species of salmon at least approaching that of production diets. For Fall run chinook salmon, however, nothing but failures have been reported. Omitting theoretical discussion, if this failure is due to the inability of chinook salmon in fresh water to ingest or otherwise put sufficient water into the stomach so that normal digestion can proceed, then the addition of water to a pellet should greatly improve its growth response.

Neal Woodall prepared seven diets by adding varied increments of water to a commercially available dry fish pellet which had a previous history of success for trout. Diet consistency was maintained constant throughout (with the exception of the first diet which was fed dry) by the addition of appropriate quantities of carboxymethylcellulose (a biologically inert binder). All diets were fed ad libitum, 3 times daily, 6 days per week. The trial was conducted at the Hagerman station. The graph in figure 8 shows the mean weight gain and conversion for the 12-week feeding trial plotted versus the total percent moisture in the diet. The lack of response shown by the last 3 points on the weight gain graph can be explained by the fact that the fish are eating as much as they can hold but that the total amount of nutrient ingested necessarily decreases as water forms an increasing portion of the bulk of the diet. The graphs on the second chart will illustrate this point. This work was presented at the Northwest Fish Cultural Conferences in Seattle, December 3, 1958, and will be submitted in greater detail for publication in The Progressive Fish-Culturist.

Some general considerations of the problems involved in fish husbandry can be reviewed in Woodall, in press.

BIOCHEMISTRY and METABOLISM

Nutritional induced effects

Nicotinic acid and sunburn:--On numerous occasions a disease called "sunburn" has been reported in fish hatcheries throughout the United States. To our knowledge this disease is considered to be a physical situation caused by too much exposure to sunlight. However, many reports have been made of a specific dermatitis irritated by sunlight in other animals due to niacin deficiency. Dr. Donald C. DeLong speculated on the close similarity of the increased susceptibility to sunburn of pellagra patients and the observations of many hatchery personnel of the sunburn lesion. W.T. Yasutake felt that histology could follow closely the development of these lesions and was willing to cooperate in a project to determine whether certain types of sunburn were nutritionally dependent. Two sets of experiments were designed in which a niacin-deficient diet was fed to chinook salmon exposed to sunlight. During the third week of feeding this deficient diet, typical lesions of sunburn began to appear. After four weeks of feeding these lots of fish were split into two groups. One was continued on the niacin-deficient diet and the other fed a complete diet. After one week of feeding the complete vitamin diet, this group of fish showed signs of recovery and after 30 days of an adequate vitamin intake showed signs of regaining nearly normal condition. Considerable histopathological evidence that a niacin deficiency was closely correlated with the sunburn lesions observed in many production hatcheries was assembled. The degeneration and necrosis of epidermis and dorsal longitudinal muscles was observed in the four-week deficient group. In severe cases the epidermal layer was completely sloughed off and many dorsal voluntary muscle bundles directly under the lesions lacked striations and were swollen homogeneous hyalin masses. A more complete analysis of samples collected during the course of the experiment and samples sent to the laboratory from outbreaks of sunburn in production hatcheries showed that, in many cases, the lesions

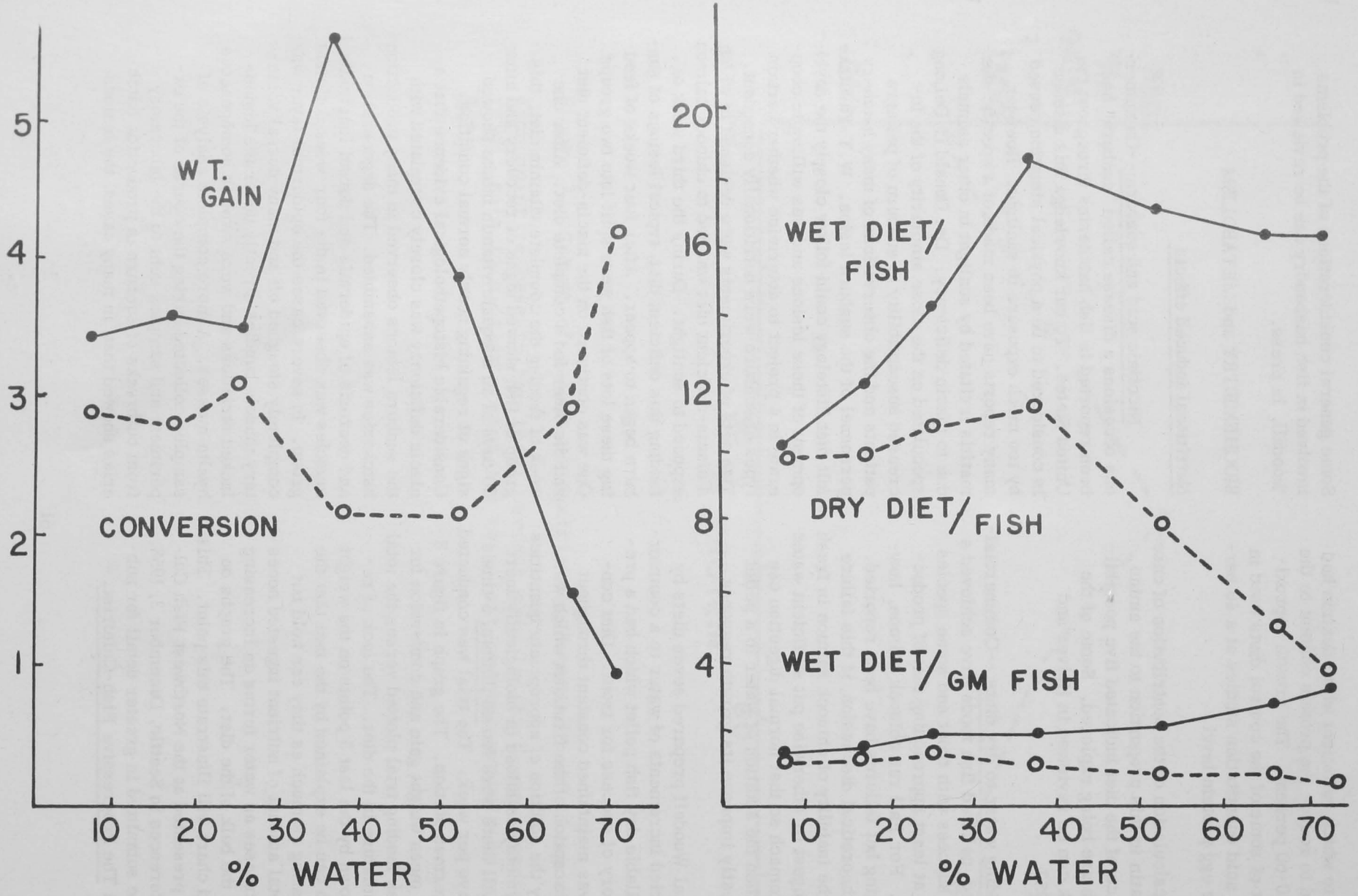


Figure 8. Growth of chinook salmon on dry diets with added moisture.

The unlabeled dashed line on the bottom of the right-hand graph represents the dry diet consumed per gram of fish. All points represent mean values for each diet at the end of the 12-week feeding period.

observed in the nutritionally induced sunburn fish were similar or identical to those found in production hatcheries. A more complete discussion of these studies can be found in DeLong, et al, 1958.

Since niacin seemed to induce a predisposition to sunburn, Dr. Donald R. Buhler thought it interesting to investigate the etiology of this photosensitization of fish. He has designed a study of the effect of a known photodynamically active compound (8 methoxypsoralin) upon fish. The material will be fed in the diet at various levels to paired groups of chinook salmon fry, one of which will be kept in the dark and the other one exposed to long wave length UV light. The pathology of any lesions which develop will be investigated and compared with those found in established cases of sunburn.

Vitamin K and hemorrhagic gills:--There are reports in the literature on hemorrhagic gill condition appearing in populations of fish without indications of a causative organism. Dr. DeLong thought for this reason it might have a nutritional origin. Since hemorrhagic diseases have been reported in other animals with a vitamin K deficiency, he induced such a deficiency in chinook salmon and searched for the appearance of any indications of hemorrhagic gill conditions. The deficiency was induced by feeding an antimetabolite, dicumarol, of vitamin K to experimental lots of fish. After four weeks on this diet the fish showed definite signs of hemorrhage, i.e. the gills were white, and even the heart had only a gray cast. However, the histological analysis by Mr. Yasutake failed to show typical signs in the gills themselves. Other general indications of a typical hemorrhagic gill condition were observed, however, and it is logical to predict that under conditions of inadequate vitamin K supply or of the ingestion of materials which would tend, either to destroy vitamin K, or act as an antimetabolite to it, certain types of hemorrhagic gill conditions would appear in the fish population. The possibilities of studying the vitamin K requirements of various species of salmon are enchanced by this finding that dicumarol can be used successfully to induce a vitamin K type deficiency in fish.

Hyper and hypothyroidism:--Dr. DeLong felt that hyper and hypothyroidism could probably be induced in fish populations. Mr. Yasutake felt that histology could serve an important tool in following the development of the specific condition by a thorough study of the thyroid gland. A set of feeding trials was designed for different lots of chinook salmon to compare growth, mortality, morphology and histology of individuals between groups to detect appearance of hyperthyroidism or hypothyroidism. A positive growth response over the controls and the hypothyroid fish was found in the hyperthyroid group. Later tests showed this apparent growth was due to morphological changes. The gross morphological changes can be readily seen in the picture in figure 9. A more complete discussion of the experimental techniques and findings are being prepared for early publication.

Enzyme systems in fish

Transaminase:--Dr. DeLong realized that transaminase could play a very important role in carbohydrate sparing action through its function known in other animals in the synthesis of nonessential amino acids. He first demonstrated its presence in heart tissue with methods of a qualitative nature using paper chromatography and crude extracts. A preparation of the enzyme was then isolated from extracts of chinook salmon heart tissue and tested with the ten indispensable amino acids to determine the rates at which these amino acids aminate. The ten indispensable amino acids for salmon aminated much slower with salmon transaminase than with rat transaminase. However, alanine and aspartic acid aminate approximately 20 times as fast as any of the individual essential amino acids.

A forced-feeding study with young chinook salmon and pure protein resulted in the evolution of approximately 60 units of ammonia whereas forced-feeding protein and carbohydrates together resulted in the evolution of only 20 units of ammonia. Two theories of carbohydrate sparing action can be suggested: (1) Carbohydrate reverses the ornithine cycle (possibly absent in salmon), and (2) carbohydrate furnishes an energy source for the formation of reduced diphosphopyridine nucleotide which will combine with ammonia and aminate a-keto glutaric acid to

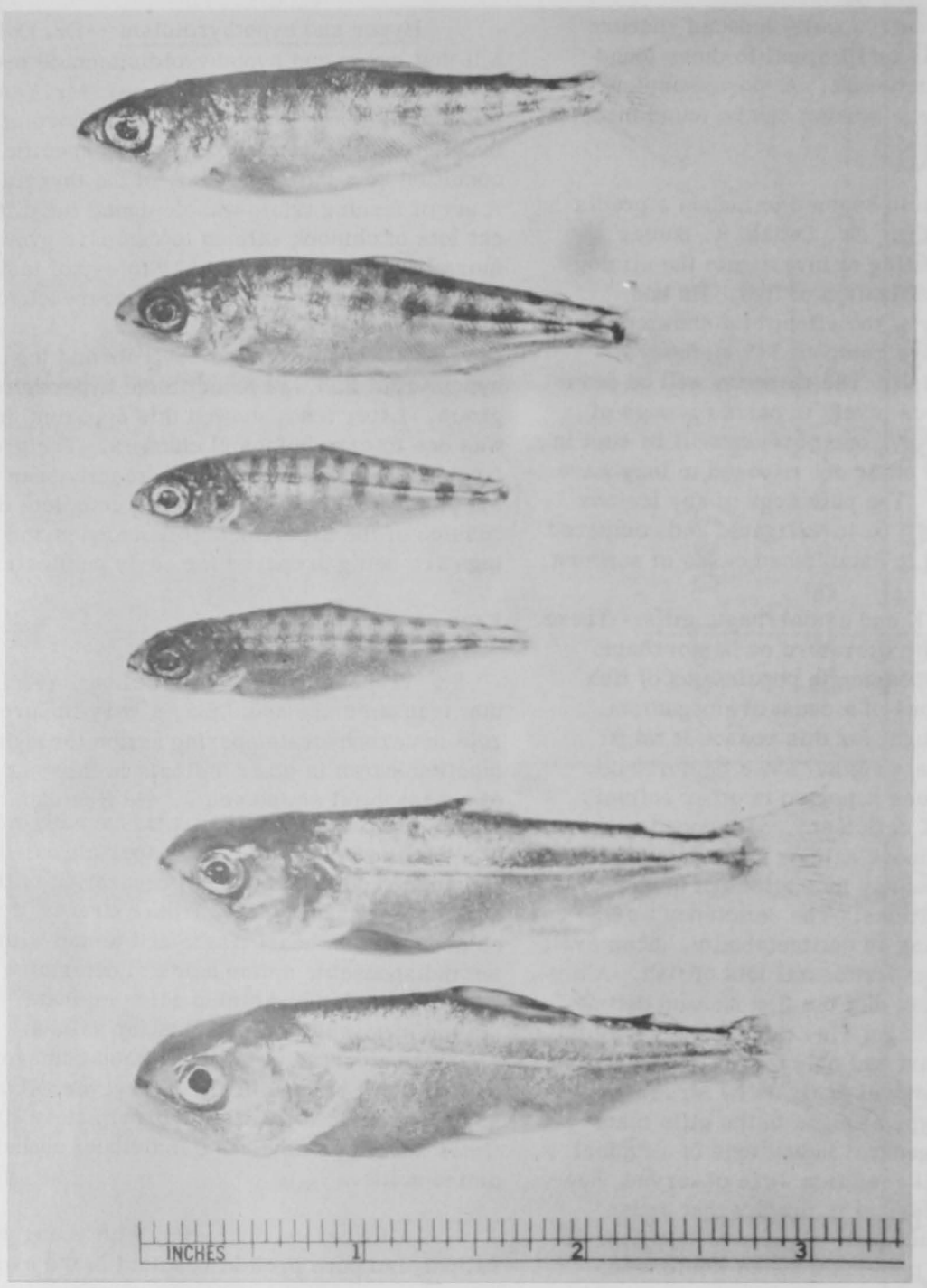


Figure 9. Hypothyroidism and hyperthyroidism in chinook salmon. The top two fish are controls fed a complete test diet. The center two show hypothyroidism and the bottom two symptoms of hyperthyroidism. Note the large heads, silver sheen and elongated opercles of the hyperthyroid fish.

form glutamic acid which is a non-essential amino acid. Therefore, evidence is accumulating that the second theory of carbohydrates sparing action is the major pathway of nitrogen sparing by carbohydrates in chinook salmon. A more thorough discussion of these experiments can be found in DeLong's thesis, 1958.

Proteinases:--Study of the digestive enzymes of an animal is one approach in understanding which types of food can be most readily utilized. A knowledge of the properties of an animal's enzymes helps us to understand its biochemical processes. Fish are interesting subjects for study of comparative enzymology because of their exceptional living conditions, especially their relatively low body temperatures. Only scattered and incomplete studies have been made of the digestive enzymes of fish. Salmon pepsin has been crystallized and characterized but little is known of the other digestive enzymes.

Dr. C. Bradford Croston's study of the digestive enzymes of salmon have so far been confined to the proteinases found in the pyloric caeca area. Phases of the work can be grouped into finding ways to detect and measure the various activities; developing methods for extracting and purifying the enzymes; and determining the property of the enzymes usually as mixtures in crude and in partially purified preparations. To be a good source of proteolytic enzymes the pyloric caeca must be collected from actively feeding salmon because spawning fish do not contain active digestive enzymes. A collection trip was scheduled to the coast to obtain suitable caeca which could then be stored in a frozen condition for long periods without noticeable change in enzymatic activity. Water extracts of these caeca contained a good supply of active proteinases which have been found assayable by known methods.

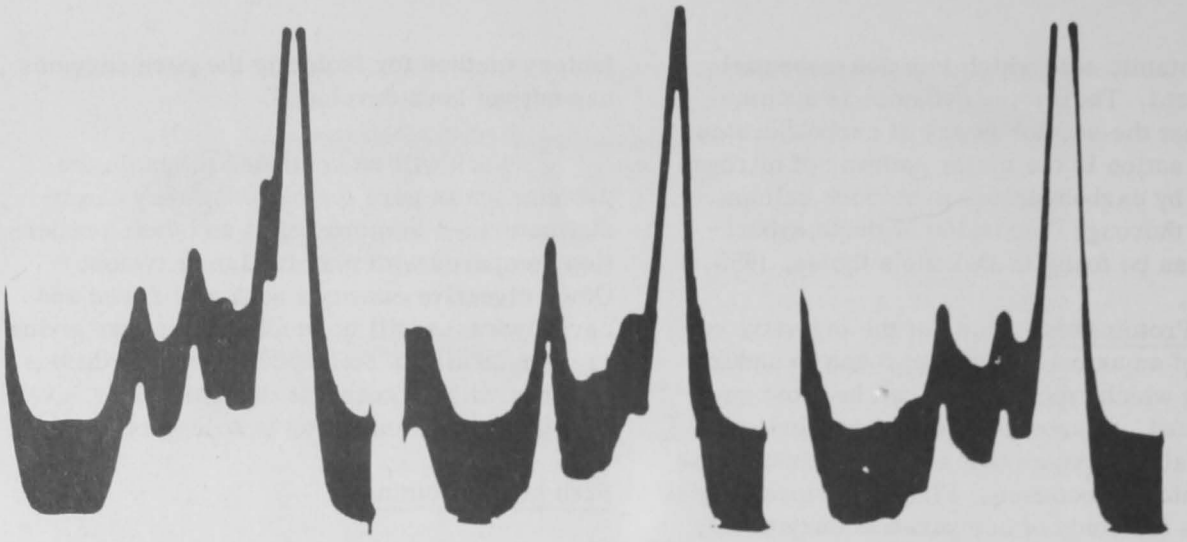
Two simple methods have been developed for obtaining protein fractions from extracts which are enriched about 10-fold in proteinases. One method salts out the protein with ammonium sulfate from clarified pH 8.5 self-digest. The second method makes use of a differential solubility at pH 6.5 and pH 4.5 based on initial solubilities at pH 4.5. Column chromatography with various materials has been studied but a satisfactory method for isolating the pure enzymes has not yet been developed.

Work will be continued to obtain the proteinases in pure form so that they can be characterized in more detail and their properties compared with mammalian enzymes. Other digestive enzymes such as lipases and carbohydrases will be studied. A paper giving greater details of the experimental conditions together with an adequate discussion of the various phenomena observed is being prepared.

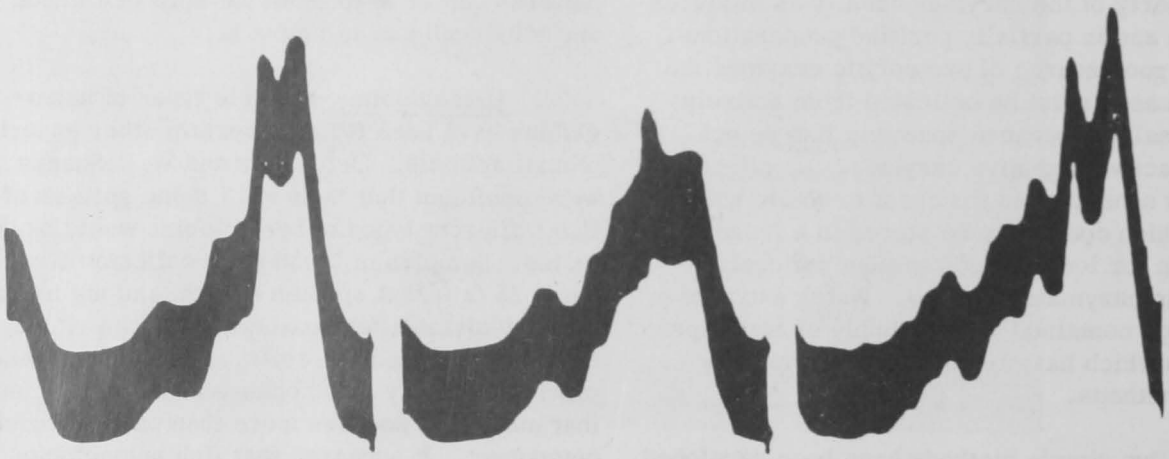
Fish blood proteins

Blood sera:--Dr. Croston conducted a preliminary survey using both paper and boundary electrophoresis on the sera of trout and salmon. This preliminary survey showed considerable variation in the number, concentration and mobility of components in the blood of individuals of the species of rainbow trout, silver salmon and chinook salmon. Though part of these variations may be due to racial differences, information was not available to distinguish between the many factors which might possibly contribute to the noted variations. The values for individuals for different species sometimes overlapped making species identification difficult. Some typical electrophoretic patterns can be seen from the sera of chinook and silver salmon in figure 10.

Hemoglobin:--Multiple types of hemoglobins have been found in certain other experimental animals. Dr. Buhler and W.E. Shanks were confident that in at least some species of fish, different types of hemoglobins would be detected. Samples of blood were collected from some 25 different species of fish, and the hemoglobin isolated after hemolysis and centrifugation of the washed red cells. Paper, starch and moving boundary electrophoresis demonstrated that many fish possess more than one hemoglobin component. It appeared that fish hemoglobins in general have an iso-electric point somewhat higher than other species. Current research is being conducted to determine the exact iso-electric point of the hemoglobins from several of these fish species. Preliminary studies are also in progress to investigate the feasibility of separating these hemoglobins on buffered ion exchange resins (200-400 mesh IRC-50). An



Electrophoretic Patterns of Chinook Salmon Sera



Electrophoretic Patterns of Silver Salmon Sera

Figure 10. Moving boundary electrophoretic patterns of serum of individual salmon.

Ascending boundaries in 0.1 ionic strength Veronal buffer of pH 8.6.

illustration of the different types found can be seen in figure 11 together with a more complete discussion included in Buhler and Shanks, in press.

Fish fats and their body use

Requirements for fats and fat-soluble vitamins:--Dr. Nicholas Nicolaides has been concerned with the requirements of fish for fats and in perfecting specialized techniques to isolate and identify the fats concerned and their specific requirements in growth and metabolism. To do this effectively, an adequate test diet composed of chemically well-characterized components was sought so that as each constituent is systematically deleted, its nutritional value could be assessed. As a point of departure the standard test diet of the laboratory using corn oil as the sole source of fat was chosen, since it is known that such a diet will maintain salmon fry in active growth for at least 20 weeks. The level of fat employed in this test diet has been 9 percent of the dry weight. Corn oil consists of at least 98 percent triglycerides of which the exact arrangement of the fatty acids in the triglyceride molecules is not completely known. However, it is known that the constituent fatty acids are linoleic 55.5 percent, oleic 30 percent, palmitic 10 percent, stearic 3 percent, linolenic 0.5 percent, and those above C_{18} plus those below C_{16} 1 percent. In order to determine what fatty acids, if any, are required by salmon fry it was felt that a synthetic corn oil could be substituted for natural corn oil. The synthetic product would consist of the triglycerides of individual fatty acids made up in the proportion that the fatty acids occur in natural corn oil. That the arrangement of the fatty acids on the glycerol molecule in the triglyceride may not be too significant can be seen from the fact that during digestion triglycerides are broken down to mono and diglycerides. If a diet of synthetic triglycerides could be found which would maintain fry in active growth for a suitable experimental period, then the requirement for each fatty acid could be determined by the systematic deletion procedure. Acids of the linoleic and linolenic "type", for example, have been found to comprise two classes of essential fatty acids for warm blooded animals. By the same deletion procedure, the requirements for the fat-soluble vitamins A, D, E and K, and thioctic acid could be determined.

However, the cost of synthetic diets of the type proposed would be rather high, since pure triglycerides of individual fatty acids range from $1.00 to $5.00 per gram. So, in order to cut down on the cost of such a program, an experiment was designed to answer the following questions: (a) Is any fat necessary? (Preliminary but inconclusive experiments done by A.N. Woodall prior to Dr. Nicolaides' arrival, indicate that it is.) (b) If so, what level is optimal for the level of other nutrients and water temperature used? If low levels of fat are required, this would greatly cut down cost of diet. (c) Are high levels of fat toxic? This question is of some practical importance since many production diets run high in fat.

An experiment designed to answer questions a, b and c, was performed during the last feeding period (winter and spring of 1958). However, the fish became infested with the protozoan, Hexamita salmonis, and no conclusive results could be obtained. A repetition of this experiment is planned for the coming months under conditions of even greater sanitation and perhaps with the possible use of the antibiotics found by Dr. Buhler and Mr. Yasutake to be effective against the growth of this organism.

Methods for lipid analysis of fish tissues:--One of the main efforts of Dr. Nicolaides has been the investigation of newly developed techniques for separation of various classes of lipids, some of these methods being of recent discovery. Gas chromatography has proved a versatile tool. This is a new, extremely efficient technique for the separation of many molecules important in lipid analysis. An Aerograph gas chromatographic apparatus was purchased, installed and tested on model mixtures. It was found to be capable of separating the normal saturated methyl esters of adjacent homologues up to C_{24}. It was also found capable of separating n-alcohols up to at least C_{20}. It has been reported that the methyl esters of oleic, linoleic and linolenic acids have also been successfully separated on polyester columns but this has not as yet been tried.

Column chromatography was also used. In recent literature it has been reported that silicic acid is capable of separating the neutral lipids from the phospholipids. Furthermore, the neutral lipids can be separated into the

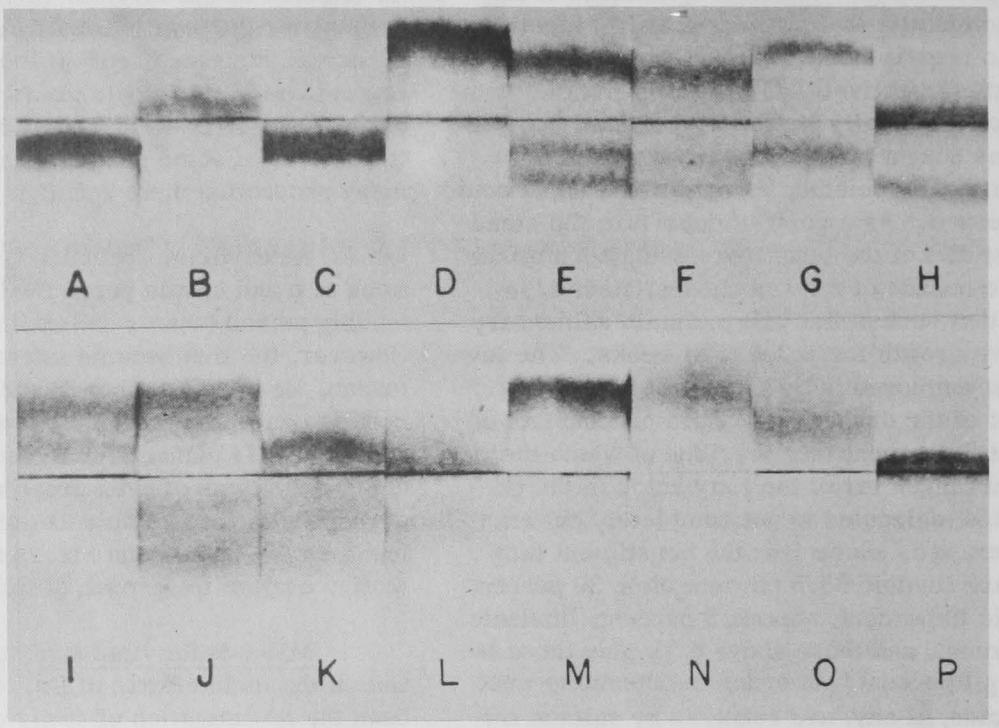


Figure 11. Paper electrophoretic separation of fish hemoglobins in Veronal buffer pH 8.8, ionic strength 0.05.

A. Entosphenus tridentatus (Pacific lamprey); B. Lepomis macrochirus (bluegill); C. Cyprinus carpio (carp); D. Human hemoglobin A; E. Salmo gairdneri (steelhead trout); F. Salmo gairdneri (rainbow trout); G. Oncorhynchus nerka (blueback salmon); H. Alosa sapidissma (shad); I. Micropterus salmoides (largemouthed bass); J. Salvelinus fontinalis (brook trout); K. Oncorhynchus kisutch (silver salmon); L. Oncorhynchus tshawytscha (chinook salmon); M. Human hemoglobin A; N. Squalus acanthias (dogfish shark); O. Ophiodon elongatus (ling cod); and P. Ptychocheilus oregonensis (Columbia squawfish).

following fractions: (1) hydrocarbons, (2) waxes + sterol esters, (3) triglycerides, (4) free sterols, (5) diglycerides and (6) monoglycerides. From previous work by Dr. Nicolaides it is known that waxes can be separated from the sterol esters either by rechromatography on silicic or if the waxes are straight chain, by urea adduct separation. Literature also reports that phospholipids, too, can be separated into five fractions. Preliminary experiments with salmon liver lipids has indicated that the neutral lipids comprise 26 percent and phospholipids 74 percent. The neutral lipids are very low in hydrocarbons and waxes but contain significant quantities of sterol esters, triglycerides and free sterols. As might have been deduced, the analysis of the phospholipids is exceedingly difficult, for, as indicated, each of the fractions are complex mixtures. Then to add to the difficulty, the fatty acid components of each of the above classes of lipids (both neutral and phospholipid) obtained from fish tissues have longer hydrocarbon chains than fatty acids derived from other sources and contain a greater number of double bonds arranged in different positions on the hydrocarbon chains. Preliminary examination of the phospholipids indicates that all 5 fractions are present in salmon liver lipids. To facilitate the identification of these and other phospholipids, model compounds are being prepared by known procedures. These are shown in table 2.

For adequate investigation of the phospholipids it is necessary to determine quantitatively certain portions of it. Since quantities available for analysis will frequently be small, colorimetric methods were used. Suitable methods and standard curves have thus been prepared for the analysis of the following phospholipid constitutents: (1) phosphorous, (2) nitrogen, (3) glycerol and (4) choline.

Extraction of fish tissue lipids:-- Dr. Nicolaides has extracted lipids from three types of fish (a) whole fry (chinook), (b) seagoing salmon and, (c) spawning salmon. The method of extraction is that of Folch et al (J. Biol. Chem. 226: 497, 1957). The lipids are sealed in glass and stored at -10° C. until analyzed. The following samples have thus far been collected: Whole fry (chinook): (1) healed fry (unfed); (2) fry fed standard test diet 9 percent corn oil, 41 days; (3) - (10) fish fed 5 levels of fat; (11) - (16) fish fed 3 levels of histidine; (17) - (21) fish fed 3 levels of arginine; (22) carcasses of fish fed production diet with ceroid pigment in the livers; (23) livers of fish fed production diet with ceroid pigment in the livers; (24) - (36) carcasses and livers of fish fed various production diets (to be used as controls for ceroid fatty liver study - see below). Seagoing salmon: Lipids were collected from blood (4 samples); testes (2); head (5); muscle (10); eggs (1). Spawning salmon: Lipids were collected from blood plasma, liver, spleen, heart, muscle.

Of spesial interest were the lipid extraction data obtained from spring chinook fry fed production diet at the Tyee Hatchery. This relatively rare salmon suffered high mortalities, in one month being as high as 13 percent. The sick fish would fall toward the tail of the raceway and refuse to eat. The livers of these fish were chocolate brown instead of the normal pink color and had almost no blood as compared to healthy ones. Histochemically, a pigment termed "ceroid" was found to be present. This pigment has also been found in other species with liver degeneration. The weight of lipid per gram of dry fat-free tissue for the livers and liverless carcasses of sick fish as well as the corresponding data for different health controls are given in table 3. The livers of the sick fish contained about 3 times as much fat as that of the healthy controls whereas the carcasses contained about 3/4 as much.

Fatty livers can be pathological or non-pathological. They are non-pathological when animals are deprived of food, for the organism mobilizes its fat reserves to the liver for oxidation. The composition of this fat is the same as that which is normally present. In pathological fatty livers this is not true. The pattern of the lipids present depends upon the type of pathology involved. In general, there are 4 types of conditions which give rise to fatty livers: (1) nutritional deficiencies preventing lecithin synthesis (necessary for fat transport), (2) competing reactions for components necessary for lecithin synthesis, (3) breakdown of the mechanism for fat removal, (4) increased mobilization of fat for various reasons.

Table 2--Reference lipids for analysis

Model Lipid	Source	Author and Reference
Phosphatidyl ethanol amine.	ox brain	J. Folch, J.Biol. Chem., 146, 35-44, 1942, and D. J. Hanahan et. al., J. Biol. Chem., 228, 685, 1957.
Phosphatidyl serine	"	ibid, ibid.
Phosphatidyl inositide(s)	"	ibid, ibid.
Lecithin	eggs	D. J. Hanahan et. al., J. Biol. Chem., 192, 623, 1951.
Lysolecethin	eggs	D. J. Hanahan et. al., J. Biol. Chem., 195, 199, 1952.
Cerebroside	beef spinal cord	N. S. Radin, Methods of Biochem. Anal., 6, 163, 1958.
Sphingomyelin	beef heart; sea anemone	Rapport & Lerner, J. Biol. Chem., 232, 63, 1958, also W. Bergmann, J. Org. Chem., 23, 1241, 1958.
Plasmalogens	beef heart; sea anemone	Marinetti et.al., J.Amer.Chem.Soc., 80, 1624, 1958 and W. Bergmann, J. Org. Chem., 23, 1241, 1958.
Cardiolipin	beef heart	A new procedure.

Table 3.--Lipid Content of Carcasses and Livers of Spring Chinook Fry.

	Carcasses		Livers	
	Number of fish	Average weight (gm)	Mg Lipid/ gm dry fat-free tissue	Mg Lipid gm dry fat-free liver tissue
Sick fish with "ceroid" pigment in livers.	107	3.02	183	725
Healthy fish from same trough as "ceroid" fish	300	14.3	290	258
Healthy fish fed production diet (Willard)	200	2.90	269	240
Healthy fish fed production diet (Tyee)	195	4.26	257	204
Healthy fish fed yeast diet (Tyee)	168	4.20	222	214

One of the main factors generating fatty livers is either a lack or improper utilization of choline which is necessary for lecithin synthesis. When this condition exists the triglyceride composition of the liver lipids increases enormously. Thus an analysis of the liver lipids of the salmon fry should give a clue as to whether such a condition exists. Fatty livers caused by lack of choline can be amended simply by supplying more choline in the diet; however, other factors than a choline deficiency may be responsible for "fatty liver" conditions.

Special methods for lipid analysis:--Before his arrival at the laboratory, Dr. Nicolaides, in collaboration with Dr. F. Laves of the Institute fur Kristallographie of Zurich, Switzerland, found that if one takes Laue X-ray photographs of single crystals of urea or thiourea inclusion compounds (adducts), there arise a series of continuous layer lines on the X-ray film. These lines give structural information about the included molecule. Fatty acids, fatty alcohols and other types of molecules making up the lipids form such adducts with urea or thiourea and are thus amenable for analysis by the technique. Two fundamental types of information can be gained by this technique: A. From the distance between the lines one can compute the length of the included molecule. This, in turn, will enable one to determine (1) the number of methyl branched groups present on the hydrocarbon chain, (2) how many cis or trans double bonds exist in the chain, (3) the molecular weight of the compound. B. From the intensities of the different orders of lines one can compute the position of substituents on the hydrocarbon chain. A complete discussion of this technique can be found in Nicolaides and Laves, 1958 and two papers in preparation. For 5 weeks in August and September 1958, Dr. Nicolaides went to Zürich to finish writing two papers on this technique. In addition to writing these papers, the groundwork was laid for the writing of three more papers and on cooperative research for the application of these techniques to other fish fats.

New metabolism studies

Carbohydrate metabolism:--Dr. Buhler joined the laboratory staff in March 1958, and immediately formulated a general research program. The study of the various biochemical transformations which occur in a given component in the diet of an organism is called the "intermediary metabolism" of that component. The intermediate metabolism of a protein or a carbohydrate or a fat or any of their chemical derivatives must be examined for each type of organism separately, since this metabolism could occur by identical or widely different mechanisms. Examination of the literature showed that little if anything is known about intermediary metabolism in fish of any of the various nutritional elements. Therefore, the intermediary metabolism of carbohydrates was first investigated because of its obvious scientific and practical importance. This study is divided into two phases. The first phase consists of a relatively short-term investigation of the carbohydrate requirements of the Pacific salmon and is aimed at giving a biochemical clue as to which carbohydrate metabolic pathways might be present in these fish. The study will also provide valuable information on practical carbohydrate nutrition. The second study involves long-term research on carbohydrate metabolism in salmon. Initially a great deal can be learned from an examination of the carbohydrate content and identity of the various tissues and organs of the Pacific salmon. This will be followed by the usual biochemical studies on intact tissues and finally by a study of the individual enzymes which catalyze the component chemical reactions. As the program on the intermediary metabolism of carbohydrates develops, other studies will be initiated on the intermediary metabolism of other nutrilites.

As the first step in establishing the carbohydrate requirements of Pacific salmon, a series of feeding experiments is currently being initiated. In this study young chinook salmon will be fed a modification of our laboratory's complete test diet containing from 0 - 48 percent of carbohydrate in the form of dextrin and, in addition, adequate supplies of vitamins, minerals, fat and protein for each group of fish. A second study carried out concurrently will compare test lots of fish grown on diets containing eight other carbohydrates in place of the normally used dextrin.

A collection trip was made to the Washington coast to obtain samples of chinook and silver salmon for the determination of amino acid, organic acid and carbohydrate content of the various tissues and organs. Live fish were transported to a temporary laboratory set up at the beach and samples rapidly taken and subjected to a hot 80 percent ethanol extraction. This rapid collection procedure was necessary in order to minimize post-mortem changes in the levels of these materials. The samples were stored at the laboratory and the various components were analyzed chromatographically as time and man power permitted.

Tracer lab:--One of the most versatile tools for studying small amounts of material and of following it through the various pathways of intermediary metabolism, is by the use of radioactive tracer techniques. Dr. Buhler has designed the radioactive tracer laboratory, which is under construction, after examination of the plans of similar laboratories and conferences with laboratory equipment manufacturers and fellow staff members. The laboratory was designed for biochemical research as well as radioisotopic research. The laboratory equipment will be completely installed by February, 1959.

Amino acid analysis:--The major concern of W.E. Shanks over the past 18 months has been to perfect techniques of amino acid assay which would be suitable for a large scale program of fish diet analysis. These techniques are necessary for full utilization of present, and constantly expanding knowledge of amino acid and protein requirements of fish. There is a duality of purpose in amino acid analysis: (1) to determine that a diet is not limiting in an essential amino acid, and (2) as a method of determining protein quality (the proportions of essential amino acids in a protein serve as one index of protein quality).

Since the 1940's, microbiological assays have largely supplanted chemical methods of amino acid assay. Microbiological methods are exceedingly simple as there is a fundamental similarity of technique whereby the mastery of a single procedure enables the analyst to assay a number of amino acids with only minor variations in technique. A series of assays of a group of experimental diets was completed. The results of these assays were reasonably accurate and will also serve as reference standards for subsequent work.

The major disadvantage of microbiological methods is the length of time to complete a series of assays. Because of this, there has been a continuing search for newer and more rapid methods of analysis. Such a method was developed in 1945 by Gale: a simple and convenient manometric technique employing enzymes isolated from bacterial cultures. The enzyme attacks the amino acid: $R\text{-}CH\text{-}NH_2\text{-}COOH \longrightarrow R\text{-}CH\text{-}NH_2 + CO_2$. The reaction is carried out in a conventional Warburg apparatus at 37° C. There are 7 specific decarboxylative enzymes isolated thus far which are commercially available. A series of experiments with three of them have been run (L-lysine, L-histidine, L-arginine). This method has proven very rapid; the entire analysis, exclusive of hydrolysis, required less than 2 hours and the results are readily reproducible. This method of assay is contemplated for routine determination of lysine, histidine and arginine.

The third method of analysis is paper chromatography. Early in the work a solvent system of buffered n-propanol which was reported to resolve 16 amino acids on a single unidimentional chromatogram was tested. Threonine, valine, methionine, isoleucine, leucine and phenylalanine were reported to be clearly resolved. These initial experiments were encouraging. However, valine and methionine were not separated. Efforts to resolve this pair of amino acids led to the development of a constant temperature chamber and a general improvement of technique. About mid-June, excellent resolution of these amino acids was obtained and a series of recovery experiments using pure amino acid standards and casein to determine the accuracy of the method was completed. The accuracy obtained was in keeping with the results of others in the field and will be satisfactory for routine quality control analysis.

Two methods of determining the amino acids present were used: (1) measurement of maximum density of the spot and (2) elution of the spot and measuring photometrically. The former method was the most rapid; the latter

appeared more accurate. Late in 1958, about October, difficulties were encountered resolving valine and methionine. It now appears that an extraneous factor, perhaps barometric pressure or relative humidity, affects this pair of acids; a series of experiments is being considered to solve this problem. Threonine, leucine, isoleucine and phenylalanine can be routinely assayed on a single unidimensional chromatogram and under certain conditions, valine and methionine as well. Experience with other solvent systems has been only fair but a suitable solvent system is available (2-Butanol-3% NH_3) for most routine work (see fig. 12). This work is discussed fully in Reference 14.

Nitrogen balance studies:--Dr. DeLong and W. E. Shanks realized that an adequate method for force-feeding small fish would have to be developed before any nitrogen equilibrium, nitrogen balance or biological value experiments could be easily conducted. Other workers have employed gelatin capsules filled with various ingredients to achieve these goals. These were tried successfully but the gelatin itself made a substantial contribution to the nutrients ingested. Another method employing a slender glass tube and plunger was tested. These tubes could be calibrated and a completely synthetic diet, deficient in a single amino acid, could be fed. By this technique, the effects of carefully controlled amino acid unbalances could then be observed. In certain cases it was postulated that the required levels of certain amino acids might be so low that a pure amino acid diet would be required which could only be fed in this manner. A series of 150 fish was force-fed by one person in about one hour. These fish were carried for a period of one month although mortalities were high due to trauma from the forced-feeding. Plans are being formulated to expand the force-feeding experiment and to develop more satisfactory methods for determining nitrogen equilibria and biological value of protein.

HISTOLOGY

Histology of defined nutritional conditions

During the last year, W. T. Yasutake made progress in the histological examination of fish samples taken under defined nutritional conditions. From the water-soluble vitamin quantitation study four groups were initially analyzed histologically for specific vitamin deficiency syndromes. These included the last two samples of the zero level and the last sample of the 1/8 level collected in the respective groups. The groups examined were deficient in thiamin, riboflavin, pyridoxine and pantothenic acid. If any pathology was present due to a deficiency, it would logically appear microscopically in the specimen most deficient in the specific vitamin. The following materials were dissected, embedded, sectioned, stained and examined:

Vitamin	Level	Last sample taken
Thiamin	0	10th week
		12th week
	1/8	18th week
Pyridoxine	0	2nd week
		4th week
		6th week
	1/8	18th week
Pantothenic acid	0	4th week
		12th week
	1/8	18th week
Riboflavin	0	16th week
		18th week
	1/8	18th week

Wild and hatchery-reared salmonids

As noted in last year's report, materials collected for the follow-up study to investigate the histological changes that may occur after the planting of hatchery-reared fish were prepared for examination. The returning adult silver salmon of the initial study, after 2 years at sea, have been sectioned and stained and approximately 95 percent of the samples have been examined. A complete report of the findings in the young silver salmon study can be found in Wood et.al., in press.

Histopathology of salmonids

More than 40 diagnostic studies were conducted for various State and Federal agencies including those of Washington, Oregon, Idaho, California, Montana, New Mexico, Michigan, West Virginia, South Carolina, New Hampshire and Maine. Some observations can be reviewed in Yasutake and Wood, 1957.

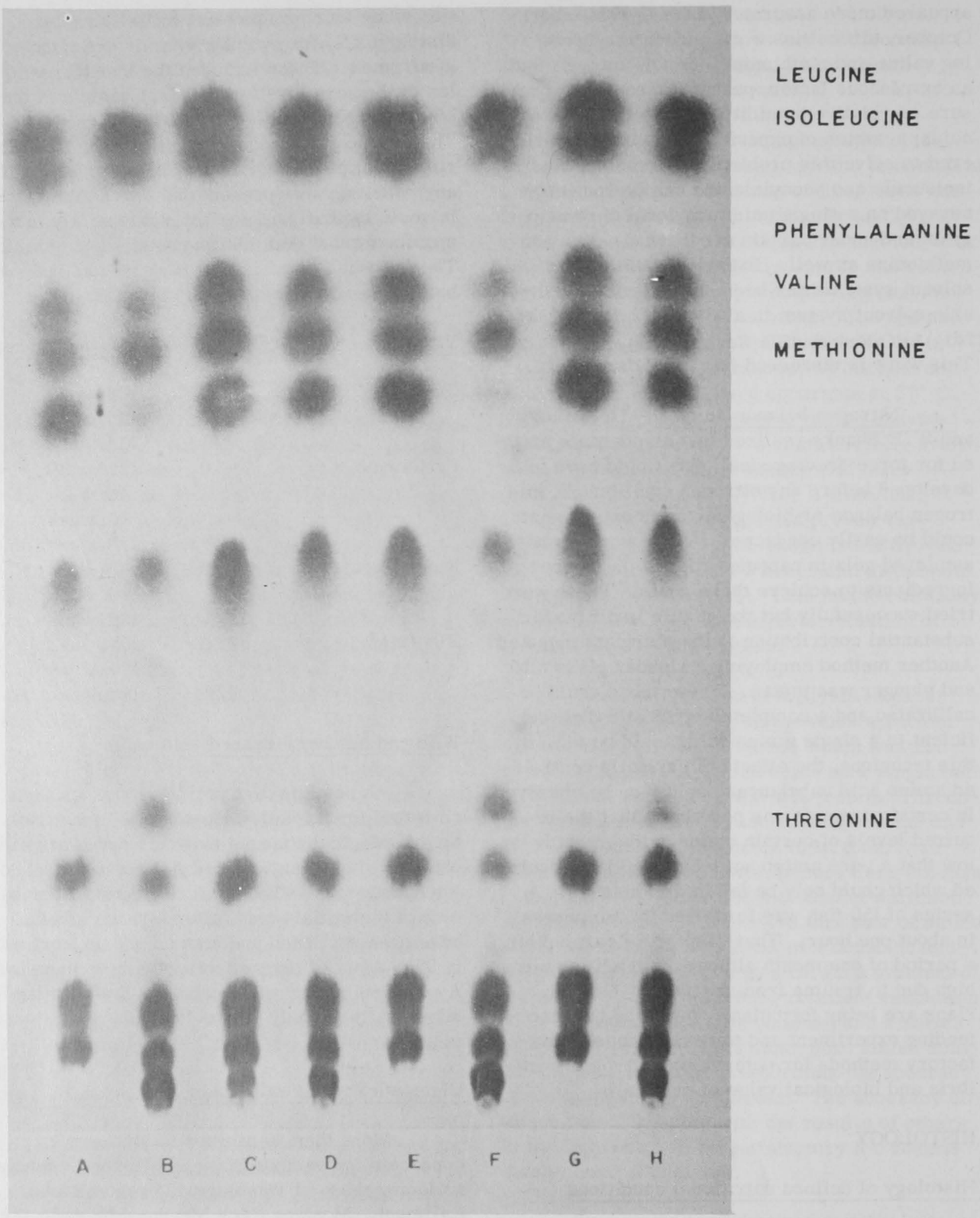


Figure 12. Resolution of six indispensable amino acids.

Paper chromatogram showing resolution of six essential amino acids for chinook salmon. A, E, and G are crystalline amino acid standards and other letters designate aliquotes of hydrolyzed whole protein.

Hematology of salmonids

With the addition of an histologist, E.F. Hesser, to the staff during the latter part of the year, steps were taken to initiate this project. Extensive literature survey on the subject is now in progress. A prerequisite to this program was to secure adequate blood smear samples of normal fish. In anticipation of this, during the collection of wild and hatchery fish for the study of normal histology, approximately 2500 blood smears were obtained from all species commonly found on the Pacific Coast. These composite samples will be used in describing the normal hematology of salmonids.

GENERAL

Quality control program

In April, 1958, a modest quality control program was initiated cooperatively with the Bureau of Commercial Fisheries, Columbia River Development Program and the Branch of Fish Hatcheries. The area of investigation for the program was the hatchery-scale testing of pelleted commercial fish foods on various species of Pacific salmon. Most of the Service's salmon hatcheries in the region were included in some phases of the testing. The laboratory's function in this stage is: (1) to analyze the various production diets used as controls at appropriate intervals throughout the year-long feeding trial; and (2) to make histological examinations of samples of fish from all lots at two-month intervals during the trials. A.N. Woodall has supervised the chemical analysis portion of the program and W.T. Yasutake has conducted the histopathology phases. John J. O'Donnell, Jr., Medical Biology Technician, has done most of the chemical analysis and E.F. Hesser, Histologist, is reading the slides for histopathological interpretations of the condition of experimental and control fish. To date 45 analyses of production diets, 36 analyses of dry diets, 62 analyses of fish have been completed and 3000 slides of fish have been examined histologically.

Although most groups showed no consistent pathology, the group of Quilcene Hatchery silver salmon fingerlings fed commercial dry pellets showed an atypical condition which may be significant. Lipomatosis (excessive fat infiltration) was found in the hearts of this latter group. Large droplets of fat lay in tissues between the heart muscle bundles. This undoubtedly will affect the normal activity of the heart. Lipomatosis is generally caused by excess fat in the body system and if the extra supply is cut off, the condition should disappear. Just why this pathology appeared only in the Quilcene silver salmon (rainbow trout samples from this station fed dry commercial pellets were also received and examined), although fishes from other hatcheries on the same dry diet did not show the fatty infiltration in the heart, is difficult to understand. Environmental factors certainly cannot be overlooked. Further samples are anticipated. It will be interesting to see whether the lipomatosis will continue to persist in the Quilcene fish.

General studies

Survival studies:--The initial survival studies together with a general discussion of the need for, and aims of such an investigation, were presented in the last annual report. The press of other urgent research problems has temporarily stalled additional work on the problem of physical stamina measurement. Much additional experimental data has been gathered, however, by A.N. Woodall, on survival against starvation. The data included "LD-50 type" determinations of food deprivation on chinook salmon from feeding trials of protein quantitation, production diets, mineral studies and others. Carcass analyses on a sample of the fish studied prior to, at intervals during (when possible), and at the termination of the period of food deprivation, have been completed. All samples were saved for a more complete elemental analysis, some of which have been completed. The compilation and evaluation of the analytical data is incomplete.

Of the many starvation tests completed thus far, one thing stood out clearly. The group of fish which, on a growth basis, were judged the best in the feeding trial, also showed the greatest survival time. In addition, and perhaps coincidentally, this group had the highest initial fat content. It should not be assumed from this that fat content per se is the sole, or even deciding factor. The fat level in the fish droped rapidly in the early stages of food deprivation and then changed little during the remainder of the period.

In many instances fish whose initial fat content was near equal showed greatly different survival times; in other instances fish of low fat showed greater survival than ones of higher fat content, but in all cases the fish which were superior on a growth basis were also superior for this type of survival. Clearly, much more data is needed on fish from diverse backgrounds before the picture is clear enough for reliance. A new tool for the evaluation of experimental fish has not as yet been perfected, but it would appear that the old one is somewhat sharper.

Chemotherapy of Hexamita salmonis:--The program of the Western Fish Nutrition Laboratory has been handicapped for several years by excessive infestation of Hexamita salmonis (Octomitus) in the experimental fish. As far as known, the only effective methods of treatment at the present time involve the use of carbarsone or calomel. However, these compounds have been shown to be somewhat toxic, especially calomel, and not too effective in completely eliminating the protozoan. Another handicap in the nutritional studies is that the treated fish frequently refuse to eat during and after administration of these two drugs. W.T. Yasutake, Dr. Buhler and W.E. Shanks, therefore, initiated a preliminary study to find a therapeutic agent which is innocuous, palatable and effective in eradicating the protozoan in chinook salmon. Twelve pharmaceutical houses graciously supplied 23 compounds. The experimental diet consisted of the complete test diet plus the chemotherapeutic agent at two different levels: (1) 0.2 percent of the wet weight of the diet and, (2) 1.0 percent of the wet weight of the diet. In the first experiment, four compounds were effective and showed little mortality. When the test was repeated at a higher level, only 3 drugs were effective but weight gain was considerably lower. A full report on the experiment is near completion and will be submitted for publication.

Construction

Water system:--An addition to the service building was constructed and an oil-fired boiler installed for maintaining 500 gallons of water at a constant temperature for the experimental hatchery. Two wells were drilled and electric pumps and pump houses installed to furnish a relatively pathogen-free clean water supply for the hatchery. An automatic gasoline operated generator was installed at the small well for emergency use during power failures. Provision was included in construction for installation of a chlorination-dechlorination system in the event the well water supplies harbor fish pathogens.

Tracer Lab:- A contract was awarded for installation of a radioactive tracer laboratory to be installed in the main laboratory building. The large upstairs room was partitioned into a "hot" lab, a counting room and a chromatography room. At the time of this report, final installation of the hood, benches, and work area is underway. The laboratory will be used as a general lab for biochemistry studies until the A.E.C. license is obtained.

Improvements to facilities:--The experimental hatchery has been thoroughly chlorinated and a wire mesh barrier erected between the hatchery proper and the main laboratory. All exterior doors are kept locked and no unauthorized personnel except those actually handling the fish, are allowed in the experimental hatchery.

Vent fans were installed in the ether extraction room and the metabolism room which is now being used for additional lipid research facilities. A large exhaust fan was installed over the muffle furnace and drying ovens.

A new and completely equipped paper chromatography laboratory was constructed in a portion of the large upstairs room. Exhaust facilities, sinks, desk and bench area were included in order that most of the chromatography could be confined to one area of the laboratory.

Personnel

Dr. Nicholas Nicolaides arrived in the laboratory in August 1957 and was placed in charge of the fat program. He came from the University of Chicago where he was employed as a lipid chemist; earlier he had returned from the institute for Crystallography in Zurich, Switzerland, where he was the recipient of two Guggenheim Fellowships in fat biochemistry.

Dr. Donald R. Buhler arrived at the laboratory in March 1958 to take charge of the carbo-

hydrate and intermediary metabolism studies. He received his final degree from Oregon State College in 1956 and then completed two years of post doctorate work at the University of Oregon Medical School.

Dr. Donald C. DeLong, who completed his M.Sc. and Ph. D. thesis research at the laboratory during the past three summers, terminated his employment here on December 2, 1957, to return to Purdue University to receive his Ph. D. in June. This completed one phase of a mutually profitable cooperative agreement with Purdue University. At present Dr. DeLong is still continuing work in fish nutrition research with A. E. Staley Manufacturing Company in Decatur, Illinois. Dr. DeLong's thesis material was on the amino acid requirements of salmon, an important and difficult field in which he made considerable progress.

Other new personnel include: Gordon C. Baker, maintenance man, in January 1958; Ernest F. Hesser, the histologist previously mentioned who arrived in June 1958; Hazel J. Jones, laboratory helper, December 1958; W. Merlin Nelson, physical science aid, January 1958; John J. O'Donnell, Jr., medical biology technician, February 1958; Wilfred Stevenson, fishery aid (laboratory), April 1958. In addition to these, three college students were employed during the summer: Sheila Y. Bernier, David H. Kapp and Michelle G. Monte.

Meetings attended:

December 4 and 5, 1957: Members of the staff attended the Northwest Fish Cultural Conference in Portland. Papers presented were: Evaluation of feeding trials, A. N. Woodall; Quantitation of amino acids, W. E. Shanks; Quantitative amino acid requirements of chinook salmon, J. E. Halver; Digestive enzymes of fish, C. B. Croston; A possible cause of sunburn in fish, W. T. Yasutake.

March 17-19, 1958: Dr. Halver attended the U.S. Department of Interior Field Executive Conference, Yosemite National Park at which time departmental problems in modern management and related problems in efficient and proper management of the complex and varied field units of the Department of the Interior were discussed.

April 1958: Dr. Halver attended the American Institute of Nutrition and other scientific meetings in Philadelphia and was elected a member of the institute. Following a brief trip to the Central Office where he presented a seminar on research progress on salmon nutrition, he was assigned to the Quartermaster Food and Container Institute, Nutrition Branch, for orientation on some of the Army's current problems and research projects in nutrition.

Dr. Nicolaides attended the Northwest Regional Meeting of the American Chemical Society and presented a paper on measurements on cis-trans isomerisms in fatty acid-like molecules with X-ray spectroscopy of single crystal urea adducts using the von Laue technique. He was invited to present papers on his work in X-ray spectroscopy at the International Congress of Biochemists in Vienna. He was able to do this because he was at the Institute for Crystallography in Zurich completing some work on analytical techniques for fish fats during September, 1958.

A. N. Woodall was able to participate in the American Fisheries Society meeting in Sun Valley, Idaho.

Dr. Halver and W. T. Yasutake attended the Pacific Northwest Fish Cultural Conference in Seattle in December, 1958. Two reports were presented, one on the protein amino acid requirements of salmon, and on chemotherapeutic treatments on Hexamita salmonis, respectively.

Drs. Buhler, Croston, Halver and Nicolaides spent two days conferring with Dr. H.L.A.Tarr and his staff at the Pacific Fisheries Technological Station in Vancouver, British Columbia. The complete research programs, progress and future plans for a complementary approach towards solving mutual fishery research projects by each research group was carefully reviewed. The next meeting scheduled for 1960 will be held at the Western Fish Nutrition Laboratory.

Mss. in preparation

Nicolaides, N., Fritz Laves and K.C. Peng.

Comparison of the Lengths of Molecules in Urea and in Thiourea Adducts. J. Am. Chem. Soc.

Nicolaides, N., and Fritz Laves

The Stereochemistry of Squalene. J. Amer. Chem. Soc.

Shanks, W.E. and J.E. Halver

Analysis of the Essential Amino Acids in Fish Diets.

Yasutake, W.T., D.R. Buhler and W.E. Shanks

Chemotherapy of Hexamita salmonis in fish.

DeLong, D.C., J.E. Halver and E.T. Mertz

Nutrition of Salmonoid Fishes. VIII. Threonine and Lysine Requirements of Chinook Salmon.

RESEARCH ON FISH CULTURAL METHODS

Salmon Cultural Laboratory
Entiat, Washington
Roger E. Burrows, Chief

Investigations in the fields of nutrition, physiology, and hatchery techniques have continued throughout the period of this report. In nutrition, our work has been directed toward the development of practical hatchery diets working in conjunction with the Willard and Cortland laboratories. In physiology, the factors affecting the sexual maturation of adult salmon and the effect of temperature on the development of salmon eggs are being explored. Under hatchery techniques, which is an extremely broad field and is connected quite closely with both physiology and pathology, more efficient trapping facilities for adult salmon have been developed, an effective algaecide for use in rearing ponds has been tested, and the factors influencing the environment in rearing ponds are being studied.

Feeding Trials

The feeding trials have been directed toward determination of the optimum protein level in the diet of chinook salmon. In 1957, the approach of substituting carbohydrate and fat for protein in the diet was used. The results of this experiment indicated that a protein level of 20 percent, wet weight, or 57 percent, dry weight, was not in excess of the optimum protein level in the diet tested. In 1958, a different approach was used in which the protein intake in a high-protein diet was reduced by the dilution of the diet with water. The final analyses of this experiment have not been made but preliminary results indicate that the protein and very probably the energy requirement of chinook salmon are higher than those of brook trout. This conclusion is based on the fact that this experiment was a duplication of the experiment conducted by Phillips at Cortland but with dissimilar results. A second phase of the 1958 experiments consisted of the feeding of the Cortland pellet mixture reconstituted with water, bound with C.M.C., and fed as a soft pellet. Although the results indicate this combination is inadequate for chinook salmon we have high hope for this method of approach to the use of dry meals in the feeding of salmon.

Both the 1957 and 1958 experiments must be regarded as preliminary in nature. In these experiments we were attempting to establish a background for the more extensive use of dry meals in the diets of chinook salmon. The use of reconstituted meals appears to offer a very

practical approach to this problem.

Acceleration of Sexual Maturation of Adult Salmon

Any program which would require the retention of adult salmon in fresh water for extended periods prior to spawning would be confronted with the problem of developing methods for reducing the holding mortality. One method of solving the problem would be to accelerate the maturation period. Both the use of controlled light exposures and the injection of gonadotrophins have been tested as means of acceleration.

In 1957, the third experiment with controlled light confirmed the 1956 trial in which it was demonstrated that shortened daily light exposure, less than the normal daylight period, would accelerate sexual maturation. A previous experiment showed that maturation could be retarded by exposure to longer than normal daylight periods. The 1957 experiment exposed blueback salmon to one hour of artificial light per day as contrasted to 9 hours in 1956. Spawning was accelerated by 14 days in 1957 and 19 days in 1956. The fish did not respond as quickly to the abruptly shortened light cycle.

These experiments demonstrated that light, not temperature, was the prime factor affecting the acceleration or retardation of sexual maturation in blueback salmon. Alteration of the daily light cycle appears to be a practical method of reducing the holding period.

In our attempts to accelerate maturation by pituitary injection we were not nearly so successful. In previous years we had tested salmon pituitaries, whole, fractionated, dried, in gelatin, and pelleted, whole carp pituitaries, and various mammalian products. Only the salmon pituitary produced a measurable response in blueback salmon. Even this response was coupled with a pronounced debilitation in the adults and high mortalities both in the adults and the eggs produced. The debilitation in the adults was attributed to a stress reaction created by an excess of ACTH in the pituitary material. In 1958, pellets prepared from three ACTH-free fractions of the pituitary gland were injected into three lots of blueback salmon. These lots reacted very similarly to the control group with no acceleration in maturation apparent.

The 1958 experiment concludes our experimentation with harmone injections as a method of accelerating maturation. The termination of these experiments is not meant to imply that this method cannot be worked out but rather that the response of salmon to controlled light exposures seems to be a much more practical solution to the problem.

Diversion and Retention of Adult Salmon

The electrical weir and holding ponds developed at Entiat have proved a good solution to the problems of the diversion and retention of adult salmon. The procurement of the sexually-mature adults when ripe without excessive handling has proved a more difficult problem. Different species and races within species respond to different stimuli on their spawning migration. Where the upstream and spawning migrations occur with a considerable interval between the problem is further complicated. Our experiences with both chinook and blueback salmon indicate that both these species and most of the races within species eventually move upstream on the spawning migration and will enter upstream traps. At Entiat we have one exotic race of large, late-spawning, summer chinook salmon which have proved particularly obdurate in responding to the upstream stimuli. Observation indicated these fish to have a predominately downstream urge.

In 1957, downstream traps were installed in the holding pond and operated during the later part of the season. These traps caught 20 percent of the mature fish from the late run. In 1958, the traps were improved and operated throughout the entire season. Much to our surprise the lower traps caught 75 percent of the spawning chinook and 35 percent of the blueback salmon. From these data it must be concluded that the upstream urge is not dominant in these chinook salmon on their spawning migration. It is apparent that downstream traps are highly desirable in any trapping operation where holding ponds are employed for the retention of the fish.

Evaluation of Rearing Ponds

This investigation was designed to determine the factors which limit production in the various types of ponds used for the rearing of salmon and trout. The first phase of these investigations consisted of an evaluation of three types of rearing ponds, determining the hydraulic conditions in these ponds and correlating these conditions with their known biological characteristics. From this investigation the desirable hydraulic characteristics of rearing ponds were defined and a new pond type, the rectangular-recirculating pond developed. The prototype of this pond has been tested in production operations for three years. These tests indicate the rectangular-recirculating pond to be more efficient than any of the pond types in present usage.

In 1957 and 1958, a closer scrutiny of the environmental conditions which limit the production in rearing ponds has been attempted. To date, these studies indicate that the factors which limit production in rearing ponds are the available oxygen and the concentration of metabolic waste products.

The oxygen available in the inflowing water, plus the aeration possible under certain conditions, limits the carrying capacity of a pond. Oxygen levels below 5.0 p.p.m. are below the normal activity level of the fish and create an unfavorable environment. Under such conditions the growth rate is restricted and, in severe cases of overloading, mortalities may result. To meet the normal activity level of chinook salmon fingerlings at water temperatures of 60° F., 0.5 p.p.m. of oxygen per pound of fish per gallon per minute of inflow is required. In raceways with conventional inflows this requirement must be met by the oxygen contained in the inflowing water. In recirculating ponds it is possible to supplement the oxygen contained in the inflow by as much as 33 percent. The sharp oxygen gradient which develops within raceways and the slight gradient in recirculating ponds makes the latter much more responsive to supplemental aeration and, therefore, more independent of the inflowing water as a source of oxygen.

The accumulation of metabolic waste products appears to be another limiting factor in the carrying capacity of rearing ponds. Our investigations to date have been confined to the measurement of concentrations of carbon dioxide, ammonia, urea, creatinine, and uric acid. Of these only ammonia and urea appear to occur in detrimental quantities.

Our investigations, to date, indicate that neither ammonia nor urea is excreted at a uniform rate and that ammonia is not an adequate measure of the urea accumulation. Urea occurs at much higher levels and for longer periods of time than does ammonia. Salmon appear to tolerate levels of urea up to 1 p.p.m., for short periods of time (one or two hours) with no measurable ill effects but exposures to rather low levels, above 0.5 p.p.m., for prolonged periods, in excess of 18 hours daily, may be responsible for bacterial gill disease. These investigations are still exploratory in nature but the data we have accumulated can be correlated with the biological conditions known to exist in the various pond types. All the evidence indicates that urea is the principal metabolic waste product which limits production in rearing ponds.

If prolonged exposures to urea concentrations of 0.5 p.p.m. are detrimental, the rapid expulsion of urea as it is excreted is highly desirable. Pond types in which the urea is allowed to accumulate can quickly create untenable environments. In raceways interchange is the factor responsible for the rapid expulsion of urea. In this pond type a sharp gradient exists between the head and foot of the pond. The fish frequent the upper end of the pond where the most favorable conditions exist. Either low flows or heavy fish loads can reduce this area of favorable environment until a large proportion of the stock are forced to exist in areas containing detrimental amounts of waste products. Under such conditions bacterial gill disease results.

In recirculating types of ponds dilution is offered as a partial substitute for interchange. This substitution is effective only if the flow pattern of the pond is such as to prevent an accumulation of waste products in the eddies with a resultant feedback into the main current. The Foster-Lucas pond is an example of the latter condition. In this pond type the urea level in the pond remains above 0.5 p.p.m. throughout the 24 hour period when water temperatures are high and even when below normal fish loads are

carried. Here the dilution factor expressed as pounds per cubic foot of water must be twice that of either the circular or rectangular-recirculating ponds if gill disease is to be controlled. Because dilution is a factor in recirculating types of ponds optimum loadings are not necessarily the maximum poundage the pond will carry if it is desired to utilize the inflowing water to maximum efficiency. For example, in the rectangular-recirculating pond type at maximum loading one gallon per minute will support seven pounds of fish but at the optimum loading one gallon per minute will support eight pounds of fish. At maximum loading a higher rate of interchange is necessary to keep the urea level from exceeding the desired maximum.

From our tests to date the recirculating types of ponds, and particularly the rectangular-recirculating type developed at Entiat, appear to be the most efficient in the utilization of the water supply. While we have regarded water current velocities within a pond as highly desirable as an aid to food distribution and self-cleaning, recent work of Canadian investigators indicate conditioning to current velocities a necessity if adequate survivals after release are to be expected. Only in the recirculating type of pond can current velocities sufficient for conditioning be economically maintained.

Development of an Effective Algaecide:

The search for an effective algaecide for the control of filamentous green algae in rearing ponds was concluded during the summer of 1957. Lignasan (ethyl mercury phosphate 6.25 percent) at a 1:1,000,000 concentration administered at biweekly intervals for a one-hour exposure proved to be an effective green algaecide. Lignasan at this level also proved effective for the control of bacterial gill disease. Its low cost, $0.70 per pound, and low toxicity (chinook salmon fingerling will tolerate a 1:1,000,000 concentration for 20 hours) in conjunction with its algaecidal and bactericidal qualities satisfied all the requirements of our search and tests of other chemicals were discontinued.

Thresholds of Normal Development of Salmon Eggs:

The determination of the effect of water temperature on the development of salmon eggs has continued. With the upper and lower thresholds of development determined for chinook salmon eggs and the stage of development necessary for this species to tolerate cold water temperatures also ascertained the investigation was directed toward similar determinations for blueback salmon (Oncorhynchus nerka). The lower threshold for development at constant incubating temperatures was demonstrated to be between 40.0° F. and 42.5° F. While this lower threshold is a duplicate of that determined for chinook salmon, it was apparent that blueback eggs were much more tolerant to cold water temperature than were chinook. At a constant temperature of 35° F. chinook eggs incur over a 95 percent mortality while blueback eggs experience an 80 percent loss. This difference in susceptibility is present also at both the 37.5° F. and 40.0° F. test temperatures, lending credence to the assumption that an actual difference in tolerance exists between the two species.

Tests are now in progress to determine the stage in embryonic development at which blueback salmon eggs will tolerate water temperatures of 35° F. The experiments have not progressed to the stage at which any conclusions can be formed. It is of interest to note, however, that blueback eggs pass through the early embryonic stages of development much more rapidly than do chinook at identical water temperatures.

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Francis, John K.	Fishery Aid	Western Fish Disease	Hagerman, Idaho
Gahimer, George D.	Fishery Aid	Western Fish Nutrition	Willard (P.O.Cook), Wn.
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Hales, Roy A.	Biological Aid (Gen.)	Western Fish Nutrition	Willard (P. O.Cook), Wn.
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Hoffman, Dr. Glenn L.	Fish. Res. Biol.	Eastern Fish Disease	Kearneysville, W. Va.
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Larson, Max E.	Fishery Aid (Gen.)	Western Fish Nutrition	Hagerman, Idaho
Lennon, Dr. Robert E.	Supv. Fish. Res. Biol.	Appalachian Sport Fish.	Kearneysville, W. Va.
Livingston, Donald L.	Fishery Aid	Eastern Fish Disease	Cortland, N. Y.
Maciolek, John A.	Fish. Res. Biol.	Eastern Fish Disease	Cortland, N. Y.
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