Mary Fukuyama

PROGRESS IN SPORT FISHERY RESEARCH 1961

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

Circular 132

PROGRESS IN SPORT FISHERY RESEARCH 1961

PATHOLOGY
NUTRITION
HUSBANDRY
PUBLIC WATERS
ENVIRONMENT

UNITED STATES DEPARTMENT OF THE INTERIOR
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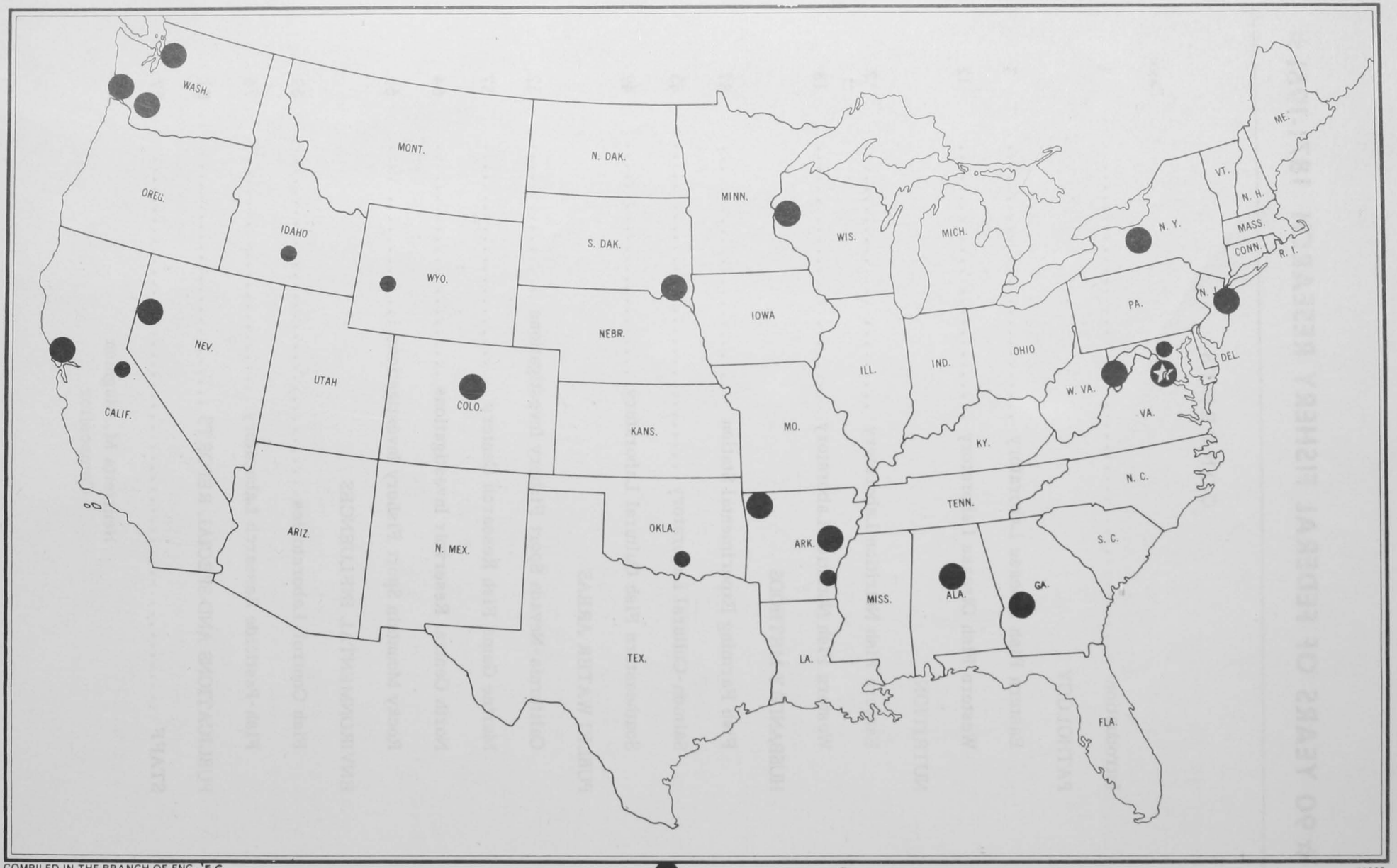
Washington, D. C.

U.S. DEPARTMENT OF THE INTERIOR

BRANCH OF FISHERY RESEARCH

FISH AND WILDLIFE SERVICE



COMPILED IN THE BRANCH OF ENG. '56

SPORT FISHERY RESEARCH LABORATORIES AND STATIONS

☆ 90 YEARS OF FEDERAL FISHERY RESEARCH * 1871-1961 ☆

INTRODUCTION

Since the Congressional Joint Resolution of 1871, establishing a Commission of Fish and Fisheries with Spencer F. Baird as the first Commissioner (as well as Assistant Secretary of the Smithsonian Institution), fishery research has been important in the resource conservation field. In this ninetieth anniversary year, a quick review of the literature of the past reveals that many of the same broad questions and problems concerned the early fishery scientists as today.

In 1873 pollution from sawdust disposal, ammonia, oil wells, gas works, and flax and hemp retting waste water was studied in relation to the well-being of fish. In the same year there were papers on fish diseases and on methods of marking fish for later identification. Before that, in 1871, the predatory nature of bluefish was observed and discussed.

The mystery of mass mortalities of fishes attracted attention in 1884 when 200 tons of fish - "mostly perch" - died in Lake Mendota, Wisconsin. About the same time, the Commission took note of the vast fish mortalities along the Florida coast in the Gulf of Mexico.

Limnological research results appeared in 1906 with Juday's papers on Lake Tahoe, California, and Twin Lakes, Colorado, followed by Birge's work on Wisconsin lakes and Pope's on Devils Lake, North Dakota.

Marine fishes and associated oceanic forms were studied and reported upon as the result of wide-ranging expeditions of the research vessel Albatross: shore fishes and deep sea fishes of the Hawaiian Islands, by Jordan, Evermann and Gilbert, for instance. Tow nets, deep sea thermometers, dredging equipment and other "oceanographic" instruments and apparatus were designed and tested beginning in the late 1870's.

Food habits, methods of artificial propagation, sense organs, habits and behavior, migrations of fresh and salt water fishes, their parasites, growth rates of hatchery fish, all were objects of study and reporting in the earliest years.

Now, ninety years later in the calendar year 1961, sport fishery research laboratories concentrated on pathology, nutrition, husbandry methods, fishes in public water areas, and problems involving environmental influences. It is not surprising, perhaps, that broad areas of research have changed but little with the passing years; problems relating to resource conservation within those areas and the scientific tools for solving them are vastly different. New and complex pollutants have been added to the scene, population and industrial growth and shifts have changed old patterns of work and leisure and eroded the freshwater environments. Sea coasts no longer have great expanses of virgin marshes and estuaries uninhabited but for shore birds and fishes and small mammals. At the same time, we have developed new attitudes, new methods, new equipment and new skills for sampling, measuring, analyzing, recording and managing.

So the research progress reports in this volume discuss fish viruses and hepatomas, enzyme systems, amino acid requirements, hematocrits and stamina tests, quality control, estuarine research, hormone stimulation, "fish farming" and "fish control", lake productivity, pesticides, electrofishing and equilibrium yields - all descendants, of course, of the work of earlier years, but new in concept and approach, if not in purpose.

PATHOLOGY

EASTERN FISH DISEASE LABORATORY
Leetown (P.O. Kearneysville), West Virginia
S. F. Snieszko, Chief

Leetown Staff
Seated - Left to right:--Juanita Collis, Millicent Quimby, Anna Basch
Standing - " " " James Warren (trainee), Ken Wolf, Howard Jackson (trainee), Alexis Knight (trainee) J. Machado Cruz (trainee), Lyle Pettijohn (Hatcheries), S.F. Snieszko, Glenn Hoffman, Graham Bullock, Clarence Dunbar

FISH PATHOGENIC AND AQUATIC BACTERIA

Hemorrhagic septicemia (infectious dropsy) and furunculosis

Schäperclaus who first described this disease called it "Bauchwassersucht" on the basis of one of the symptoms. Since death usually occurs after systemic infection and associated hemorrhagic condition, the name of hemorrhagic septicemia recommended by Spitchakoff in Poland is preferred.

The bacterium which causes this disease is now called Aeromonas liquefaciens. It is a common water saprophyte which frequently causes great mortalities among fishes and frogs. Recently this organism was isolated from diseased humans and for this reason this bacterium is being investigated vigorously over all the world. Since it is similar in many respects to enteric bacteria, Pseudomonas and Vibrio, methods of its reliable and fast identification are of utmost importance in human and veterinary medicine.

During the past year methods for isolation and identification of this bacterium were studied and described. The results were published in two papers. One of these is a schematic outline for identification of fish pathogenic bacteria prepared by Graham L. Bullock and published in The Progressive Fish-Culturist. This outline has provoked great interest among fishery biologists, bacteriologists, and veterinarians.

For the occurrence of an infectious disease three factors are required: pathogen, host and proper environmental conditions. Hemorrhagic septicemia is a common and important disease of fishes but investigators agree that infections seldom occur under experimental conditions unless the pathogen is injected into tissues. For this reason experiments are being performed to find conditions conducive to infection. In experiments performed with brook, brown, and rainbow trout, fish infected by injection became diseased and died at 54° and 64° F., but not at 44° F. Partial success was obtained by abrading the skin and placing fish in aquaria with water containing A. liquefaciens. Temperature had the same effect on infection as in injection tests.

It is relatively easy to isolate A. liquefaciens and A. salmonicida from diseased fish since the presence of other bacteria is unlikely. Isolation of these bacteria from pond water is much more difficult since other bacteria are present in abundance. Availability of selective media would be of great value. Up to this time 55 organic and 7 mineral compounds were tested for this purpose but without success for inhibition of coliform bacteria commonly present in polluted water, such as pond water.

In the therapy of bacterial diseases various chemotherapeutic agents are used. They are seldom bactericidal and usually bacteriostatic. Bacteria often become drug resistant. The most recent approach to chemotherapy is by supplementing drugs with antimetabolites affecting synthesis of nucleic acids. Properly selected drugs and nucleic acid antimetabolites as some purines and pyrimidines greatly enhance the action of drugs. Work is being carried out on antimetabolites for A. salmonicida and A. liquefaciens.

Corynebacterial kidney disease

Until recently, Corynebacterial kidney disease has been a significant mortality factor at the National Fish Hatchery, Berlin, N.H. Admittedly, the risk of inducing drug-fast organisms by long-term, low-level drug dosage is great. The possible advantages of trial prophylaxis were considered to outweigh the risks involved, and a year's trial of prophylactic feeding of sulfadimethoxine was performed by Superintendent MacKinnon of the Berlin station.

A series of three raceways of brook trout fingerlings received the drug at the rate of 2 gm/100 lb. fish/day; three raceways were fed a 1-gm level; and three raceways were not treated and served as controls.

As far as could be determined from bacteriological examination of pre-experimental mortality, kidney disease was not present in the fish at the start of prophylaxis. Mortality throughout the experiment was unusually small, and kidney disease did not occur in any fish which died among the treated lots. Sixteen control fish which died were examined bacteriologically, and the bacterium was identified in two of them. Mortality during freeze-over and loss to predators are unknown variables.

Fortunately for fish production and unfortunately for the purpose of the experiment, kidney disease occurred at such a low incidence that significant conclusions cannot be drawn from the experiment; the data were too few. The data, however, do not contradict findings on the West Coast that sulphonamide prophylaxis can be achieved. Cost is high, and the extent of the risks involved remains to be seen.

VIRAL DISEASES

Infectious pancreatic necrosis (IPN)

Infectious pancreatic necrosis of trouts continues to be a problem in hatcheries, and reports of outbreaks are increasing. This may reflect a true increase in epizootics or an apparent increase due to better recognition of the condition. At present accurate diagnosis is largely dependent upon histological examination. The purpose of continuing this research is to develop methods of serological diagnosis.

Adult rainbow trout were branded, bled, then inoculated with 2 X 10^6 $TCID_{50}$ (Tissue Culture Infective Dose) of virus. Sera were separated and preserved at -20°C. After three months the fish were bled again and the second or post-inoculation sera separated. Log dilutions of virus were prepared and different dilutions of virus were mixed with equal amounts of the sera and incubated for an hour. The mixtures were then inoculated into uniform cultivations of the RTG-2 cell strain, 5 tubes being used for each mixture of 10-fold dilution of virus and of undiluted serum. By examining each series of tubes for viral effect in the days that followed, it was possible to calculate the end point or titer (relative ability to neutralize virus) of the pre- and post-inoculation sera.

Most of the 20 fish in the experiment possessed a preinoculation serum which neutralized at least some virus. The nature of the neutralizing substance was not determined; it may have been non-specific or it may have been specific, that is - antibody. All of the post-inoculation sera showed a gain in titer or ability to neutralize virus. Since the sera of each fish were tested simultaneously under identical conditions, the pre-inoculation titer could be subtracted from the post-inoculation titer in order to obtain the absolute gain. In this way non-specific neutralization may be ignored and the gain spoken of more firmly as antibody. All fish showed a gain in titer from a fraction of a log to over four logs, and by generally accepted standards, two-thirds of the fish showed statistically significant rises - greater than a 1.3 log rise.

Sera of known titer can now be used for identifications and for other serological techniques.

When monolayer cultures of RTG-2 cells are inoculated with high dilutions of IPN virus they often display CPE (Cytopathic Effect) at but one or two foci. This phenomenon is a result of infection at one or two sites - presumably from one or two infective viral particles. As seen in figure 1, such foci are first visible as tiny

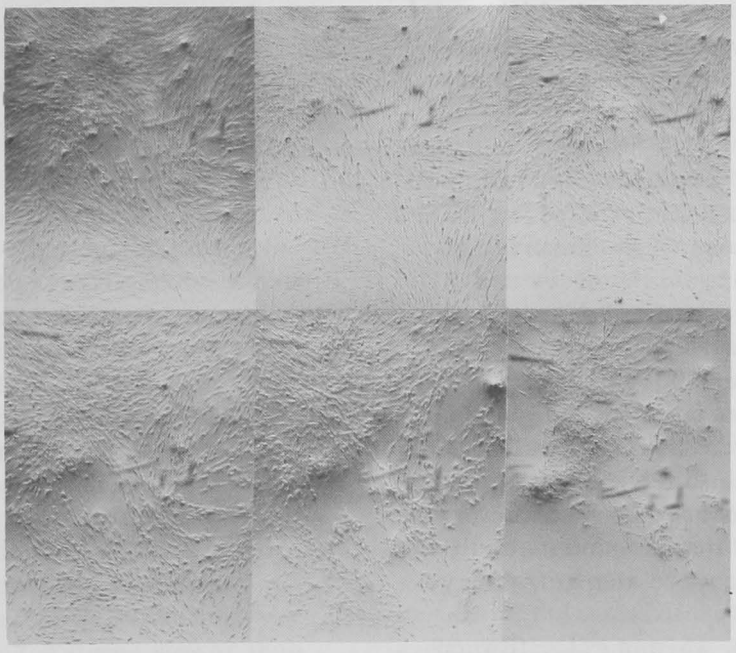


Figure 1:--RTG-2 cells in monolayer culture infected by IPN virus and photographed by oblique light to show sequence of cytopathic effect. Series represents passage of 23 hours from time focas was first observed (upper left). Degeneration is evident as contraction and necrosis within focus, but rest of sheet is normal and dividing cells are visible in the lower left corner. Necrosis proceeds in frames (left to right, top to bottom) taken at 3, 7, 11, 15 and 23 hours after the first. Scratches on glass occur as a repeated landmark in each frame of series and thus assure correct identification.

areas of necrosis in an otherwise intact sheet of normal cells. As time passes the area of necrosis enlarges, and as more virus is produced the entire sheet is infected and eventually destroyed.

Circumstantial evidence supports an hypothesis of egg transmission of IPN. In order to test this hypothesis, eggs from a suspect source were preserved periodically during incubation at the Leetown National Fish Hatchery. When samples were homogenized, filtered, then inoculated into cultures of RTG-2 cells a presumptive identification of IPN was obtained from eggs and fry preserved on the last day of hatching. Samples of eggs preserved earlier in development did not yield virus.

The work was resumed this fall. Twelve female brood stock from a suspect source were tested and a filterable agent which produced characteristic CPE in RTG-2 cells was isolated from at least one-third of the fish. Since not all cultures from each fish were infected, it was concluded that the agent was present in small numbers. When large fingerling rainbow trout were inoculated with the agent some died but evidence of IPN was equivocal. The work will be repeated with more susceptible brook trout of smaller size.

Nine strains of IPN virus have been collected and all proved capable of infecting RTG-2 cells with resulting gross CPE. Surprisingly, primary cultivations of goldfish ovary and of bluegill liver also were affected and showed at least transient, but more commonly permanent CPE. Though not tested with all strains of the virus, a line of rainbow trout fry cells and cultivations of rainbow trout hepatoma cells have also proved susceptible to IPN. Thus far mammalian cell cultivations have been refractory, and through the period it was found that cultivations of bullfrog tongue were also refractory.

A short, silent, 16-mm color film on recognition of IPN was prepared in collaboration with Lyle Pettijohn.

Viral lymphocystis disease

Lymphocystis disease of fishes is a unique viral infection that produces tumor-like growths composed of cells which are tremendously enlarged; infected cells may attain a size of 1 or 2 millimeters. The disease is well known to fish culturists and to fish pathologists but little is known of the virus itself and control measures are largely unexplored. Seasonal periodicity of infections of wild and hatchery populations makes continuing research difficult. Experimental transmission has been reported but the more successful methods have not been exploited and new experimental work has not appeared during the last 10 or more years.

The purpose of this investigation was two-fold - first to determine a method of routine transmission of lymphosystis in fishes amenable to laboratory conditions, and second, to determine additional attributes of the virus. The investigation was successful. Using methods of implantation and injection of homogenates, lymphocystis was transmitted to bluegills, green sunfish, and largemouth bass (fig. 2); but goldfish could not be infected. Bluegills proved to be easily maintained in the laboratory and were susceptible at 12.5° and at 25°C. At 12.5°C. there was no evidence of fish-to-fish spread, nor seasonal change in susceptibility, and stocks of experimentally infected fish have been maintained continuously for over two years.

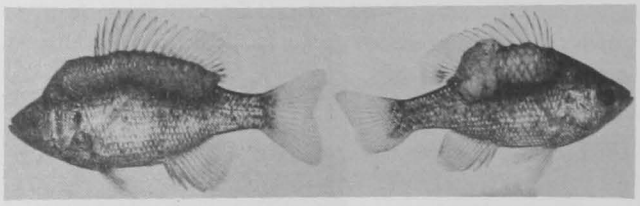


Figure 2:--Bluegills (Lepomis macrochirus) experimentally infected with lymphocystis disease. Fish on left received a subdermal inoculation with a bacteria-free filtrate, and the fish on the right received an implant of tumor tissue. Individual lymphocystis cells are visible in the posterior of the tumor on fish at the right. At death this tumor constituted 24 percent of the weight of the victim.

The virus was, for the first time, clearly demonstrated to be filterable (Millipore HA), glycerol sensitive and ether-sensitive. When maintained at -20°C. the virus remained viable for 20 months but apparently was inactivated by partial thawing and refreezing. The ability to endure desiccation was confirmed.

Inoculations of fish cell cultivations have thus far failed to produce the typical lymphocystis cell in vitro.

FISH TISSUE CULTURE

Fish cell culture

The RTG-2 (rainbow trout gonad) cell line is a continuously cultivable line of fish cells which is important because of it susceptibility to the virus of trout pancreatic necrosis The line was isolated in this laboratory two years ago and since then has been subcultivated over 50 times.

Cell cultivations from warm-blooded animals require frequent attention, but those from cold-blooded animals are not at all demanding. At 12.5°C. incubation the RTG-2 cell stock cultures are transferred but once each 2 months and intermediate feedings are not given. At 4°C. even less attention is required; test cultures have been maintained for nearly a year without attention, and mitosis continued for at least 9 months. No doubt reflecting the temperature tolerance of the intact animal, the cultivated cells' activity is affected by environmental temperature. As shown in figure 3, the greatest activity occurred at 24°C, a temperature near the thermal death point. Cultures at 18°C. required about 1.4 times as long to equal the amount of protein synthesized and glucose utilized, and at 12.5°C. approximately 2.4 times as long was required. At 4°C. protein synthesis was 3.5 times slower and glucose utilization 7.2 times slower than occurred at 24°C.

The reproduction of viruses is inseparably tied to the metabolism of host cells, and susceptibility of RTG-2 cells to the virus of pancreatic necrosis (IPN) extends from 4°C.to at least 26°C. As would be expected from the

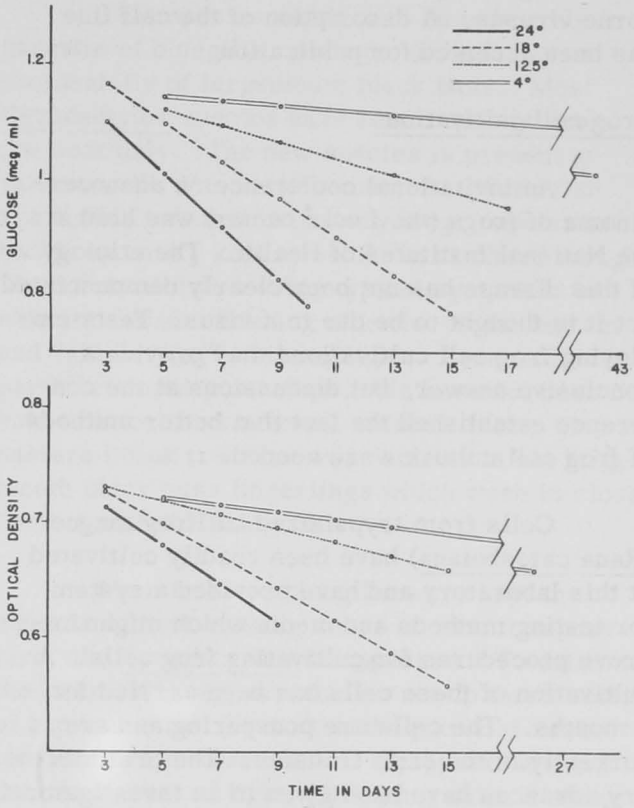


Figure 3:--Comparative metabolic activity of RTG-2 cell cultivations at different temperatures. Culture medium was systematically analyzed to determine amount of glucose remaining (upper curves), and cells of same cultures were used for determining comparative synthesis of protein (lower curves). The bromosulfalein method of protein determination shows a decrease in optical density with increasing protein. Results were treated by least squares method to obtain the points shown.

results of the metabolic studies, viral effects are much delayed at low temperatures and much accelerated at the high temperature. Maximal IPN virus production in this cell line has been determined at 19°C. and $10^{8.5}$ $TCID_{50}$/ml (Tissue Culture Infective Doses) has been produced in monolayer cultures. Identical results have been obtained by workers at the University of Miami.

The cell line has been released to other laboratories for research on fish viruses and for testing as a possible host cell for arthropod-borne viruses. A description of the cell line has been accepted for publication.

Frog cell cultivation

An invitational conference on adenocarcinoma of frogs (the Lucké tumor) was held at the National Institutes of Health. The etiology of this disease has not been clearly demonstrated but it is thought to be due to a virus. Tests employing frog cell cultivations may provide a conclusive answer, but discussions at the conference established the fact that better methods of frog cell culture were needed.

Cells from trypsinized bullfrog tongue (Rana catesbeiana) have been readily cultivated at this laboratory and have provided a system for testing methods and media which might improve procedures for cultivating frog cells. A cultivation of these cells has been carried for 6 months. The cells are prospering and are currently in the sixth transfer. The preliminary advances have been given to an investigator at Johns Hopkins University where the adenocarcinoma is being studied.

The cultivation has value in fish pathology because the cells have been found to be refractory to IPN.

Physiological saline for fish

Physiological fluids are essential to cell culture. Early comparative work showed that trout cells found conditions more favorable in mammalian-type salt solutions than they did in existing salt solutions intended for frogs or fishes. In practice, most freshwater teleost cell culture has employed unmodified mammalian-type solutions and media. The analyses of Phillips and the staff of this laboratory showed that the inorganic constituents of brown trout and of human blood were present in very similar quantities. This information served as an explanation for the compatibility of freshwater teleost cells in mammalian-type media. The data were used to formulate a physiological saline specifically intended for brown trout and probably suitable for many freshwater teleosts:

Composition of Cortland Salt Solution

NaCl	8.25 gm
$CaCl_2.2H_2O$	.23 gm
KCl	.38 gm
$NaH_2PO_4.H_2O$	.41 gm
$NaHCO_3$	1.00 gm
$MgSO_4.7H_2O$	.23 gm
Glucose	1.00 gm
Water	1000 ml

Substantiating the theoretical suitability of the proposed "Cortland Salt Solution" is the fact that the RTG-2 cells can be grown in a medium consisting of 90 percent Cortland Salt Solution, 5 percent fetal calf serum, and 5 percent whole egg ultrafiltrate.

FISH PARASITOLOGY

Myxosoma cerebralis (Myxosporidia) (Blacktail, whirling disease) of trout

This is an important and widely distributed disease of salmonid fishes, rainbow trout in particular. It has now been found in two of the northeastern States. It has been difficult, if not impossible, to control the disease at affected hatcheries and impossible to produce an experimental infection under laboratory conditions. Therefore, further experiments were carried out with six lots of 30 - 75 early feeding rainbow fry. Trout were placed in aquaria at 54°F. Suspensions containing many spores were prepared by homogenizing infected yearling trout with the Waring blendor or macerating with mortar and pestle. This material was added to the aquaria just after the fish had begun to feed. Another lot was fed such a suspension for 6 days. All fish were kept in the contaminated water for 5 to 14 days and then transferred to running spring water. Whirling was seen in a very few at about 2 weeks but it was not possible to verify any infections in tissue sections or otherwise. Further work on this parasite is postponed pending the outcome of experiments with a similar Myxosoma in bluegills which is more readily available.

A new species of Myxosoma in bluegill sunfish

This myxosporidian resemble Myxosoma cerebralis and was found in 1- to 6-month-old bluegills from the Leetown hatchery and a nearby farm pond. It is also a cartilage parasite and we have found it in the gill arches, isthmus, cranium and base of the anal fin but not in the auditory capsule. The lesions also resemble those of M. cerebralis histologically, but unlike it they disappear when the fish are about 5 to 6 months of age. It causes no symptoms comparable to those of trout whirling disease. There were many mortalities in the 1960 and 1961 lots but the cause could not be ascertained -- the fish were also infected with Gyrodactylus and three other Myxosporidia. Many Myxosoma cysts were fed to 24 small bluegills from a non-infected source over a 35-day period but to date no experimental infections have been found.

In an effort to find a satisfactory disinfectant for Myxosporidia, Mr. Golding, a trainee, tested several reagents for polar filament extrusion, which, previous to fish ingestion may render the spore non-infective. Sodium hydroxide (1.5 - 2 percent) caused polar filament extrusion of 67 - 93 percent of the spores in 20 - 30 minutes. Roccal, 5 percent benzalkonium chloride, caused extrusion of 6 percent in 20 minutes. Sodium hypochlorite, 0.1 - 1.3 percent, caused some extrusion and the spores gradually dissolved in less than one hour. The following showed no polar filament extrusion capability at all: acetic acid, 10 - 50 percent; acetone, 50 percent; aminotriazol, 5 percent; ammonium hydroxide, 3 percent; calcium cyanamide, 10 percent suspension; calomel, 2-1/2 percent; carbarsone, 2-1/2 percent; formalin, 50 percent of commercial formalin; furaxolidone, 2 percent; glycerine, 10 percent; hydrogen peroxide, 0.3 percent; household detergent, 4 percent; malachite green 0.0025 - 2.5 percent; merthiolate, 0.01 percent; picric acid 50 percent; potassium permanganate, 5 percent; PMA (pyridylmercuric acetate), 5 - 10 percent; quinine sulfate, 1.25 percent; trypsin, 0.024 grams in 1 - 5 percent sodium bicarbonate; sodium chloride, 3 - 10 percent; trisodium phosphate, 0.1 - 10 percent; Tween 40, 0.5 - 10 percent; versene, 0.01 - 50 percent.

A new species of Gyrodactylus of bluegills

This species appears to be primarily a parasite of bluegills, perhaps secondarily or accidentally of largemouth black bass. Most Gyrodactylus species have been reported from one host only. The new species is present at Leetown, W. Va., and Lamar, Pa. Pond fish are lightly infected but a very large population builds up on fry kept at 54°F. in hatchery troughs. The infected fish develop typical symptoms of skin irritation--flashing sidewise to the bottom and "shimmying" while upright. Eventually a partial immunity develops. We have not been able to infect brown trout, rainbow trout, or eastern brook trout but were able to infect largemouth black bass fingerlings which were in close contact with the bluegills.

When infected bluegills were kept in aquaria without running water at room temperature, the Gyrodactylus disappeared. To determine the cause of disappearance we set up three series of experiments: (1) darkened aquaria at room temperature to determine if disappearance was due to increased light; (2) spring water running into the aquaria to determine if it was waste accumulation and higher temperature; and (3) aquaria without running water set in the 54°F. troughs to determine if it was waste accumulation. To summarize--the Gyrodactylus flourished at 54°F. in "standing" as well as running water but disappeared at room temperature in light or dark, running or standing water.

Dermocystidium sp.

The taxonomy of this parasite is still obscure. Some investigators believe it is a fungus, for example, Dermocystidium marinum from oysters, and others believe it is a protozoan belonging to Haplosporidia.

Opaque white cysts about 5 x 1 mm in size containing Dermocystidium spores were submitted by Mr. MacKinnon of the Berlin, N.H., National Fish Hatchery. In November we found 2 Dermocystidium cysts on the caudal fin of a 3-inch bluegill from a pond near Leetown. These are the first eastern United States records of this parasite and the first record from the fins

of North American fish. Previously Dermocystium has been reported from gills of western salmonids.

Peritrichous protozoa

Specimens of Scyphidia and Epistylis have been examined from hatcheries at Berlin, N.H.; Sulton, Mass.; Pisgah Forest, N.C.; and Montebello, Va. The morphological studies have not been completed.

Dr. Jiri Lom, Czechoslovakia, is hoping to monograph the trichodinids. We plan to assist her with North American material. She will be happy to receive material from other North American sources.

Life cycle and pathogenicity of Phagicola sp. and Ascocotyle sp. (Trematoda: Heterophyidae)

Mortalities of Gambusia affinis submitted by Dr. Cope were determined to be caused by blockage of the conus arteriosus due to trematode cysts inside that organ. One species (Phagicola sp.) was successfully raised to adult in baby chicks. Species determination has not been made. Laboratory-reared snails have been exposed to the miracidia. The Ascocotyle species metacercaria did not develop in the chick, presumably because the extremely thick, tough cyst wall was not digested in the chick. The infected fish were taken from a hot springs pool in Utah--temperature 104 to 120°F. We have no previous record of fish trematode development at that temperature.

Ichthyophthirius episode

A small lot of infected 1- to 2-inch bluegills from Lamar, Pa., were held in a trout trough at Leetown at 54°F. The following observations were made: First day - very few parasites present; fifteenth day - many parasites could be seen with the naked eye in the epithelium of the fish and the fish were suffering typical skin disease symptoms and not feeding; seventeenth day - infection at its peak, some fish literally covered with parasites; twentieth day - sloughing of whitened mucus and/or epithelium, not as many parasites present, fish appeared more normal; twenty-sixth day - a few still had patches of sloughing epithelium, very few parasites; twenty-seventh day - no parasites could be seen, some fish feeding. There were no mortalities during the entire episode. These observations indicate that the Lamar strain of Ichthyophthirius is a cold-water form and that the bluegills develop an immunity in about two weeks.

HISTOLOGY AND HISTOPATHOLOGY

Hepatoma of rainbow trout

An extensive search for secondary metastatic tumors in fish with advanced hepatoma has been conducted. To date 39 4-year-old rainbows have been critically examined for abnormal growths. Seven tumors representing true metastases have been positively identified. Five were located in the gills, one in a spleen, and one in a mesentery lymphatic channel. One gill metastasis, microscopic in size, was lying wholly within a blood vessel. The remaining gill tumors were grossly visible as small light-brownish to white nodules growing on the gill filaments. The tumor in the spleen was visible to the unaided eye as a white spot about 1 cm in diameter. Grossly the spleen appeared normal excepting the white nodule, but microscopically it was filled with very small groups of tumor cells. The larger nodule was composed of cells indistinguishable from liver cells.

Microscopically the gill tumors consisted either of liver-type cells or a mixture of liver-type cells and bile duct elements. When a mixed tumor was found in the gill, the primary liver tumor was also a mixed tumor.

The invasive nature of rainbow trout hepatoma was evidenced by extensive adhesions in the visceral organs. Masses of connective tissue literally binding the viscera into a single large mass, served as a substrate for the tumor cells to multiply and grow from the liver into and around the remaining organs. Several masses of tumor cells were observed growing on the surface of the stomach, cecae, intestine, and in the pancreatic tissue. This laboratory collaborated with the Department of Pathology of Ohio State University in electron microscopy; inclusion bodies indicating a possible virus etiology of this disease were not found.

Blue slime in brook trout

For the past several years a "blue slime" occurred in the yearling brook trout at Leetown. This is a condition in which a bluish film occurs along the dorsal side of the fish extending from the head posteriorly behind the dorsal fin. The bluish color is due to an increased number of mucus cells, brought about by extensive proliferation of the epithelial cells. Proliferation is so rapid and extensive that the dermal layer is pulled into folds. This in turn affects the position of the body scales. Normally the scales are lying in a horizontal plane to the body surface, but under the conditions described above they are nearly vertical to the body surface.

A type of a blue-slime disease has been reported as a nutritional disease due to a deficiency of biotin and pantothenic acid. However, since this condition appears annually at Leetown and its appearance corresponds to the time when gonadal development is taking place, hormonal etiology is implicated. The nature of the lesion also suggests the possibility of a virus infection. Materials from fish with blue-slime are presently being studied with the electron microscope at Ohio State University.

DIAGNOSTIC PROCEDURES AND NON-INFECTIOUS DISEASES

Hepatoma of rainbow trout

In research on hepatoma and in particular in attempts to reduce the incidence of the disease by selection and breeding, diagnosis of hepatoma by blood examination may be of value. One phase of such an investigation was just completed on a lot of rainbow trout 3.5 to 4 years old with a high incidence of hepatoma. The presence and stage of the disease was quantitatively established by weighing the fish and their livers and by liver examination. The following observations were recorded:

1. In trout without hepatoma the weight of the livers was 1 to 1.25 percent of the body weight. In very advanced cases the liver weight was greater than 10 percent of the body weight.

2. Incidence of hepatoma and the relative weight of the livers was greater in females.

3. Trout with hepatoma weighed less than normal trout in relation to body length.

4. The following parameters increased with the increase of liver weight: hematocrit, total serum protein, serum albumin, serum globulin and total serum cholesterol. Statistically indicative were the values for hematocrit, serum protein and serum cholesterol. Statistically significant were the combined values for hematocrit and serum protein as well as for serum globulin.

The albumin to globulin ratio decreased with liver weight increase.

Serum albumin and globulin patterns as determined by paper electrophoresis were greatly influenced by advanced hepatoma. A report by John Miller (trainee), S. F. Snieszko, and C. Atherton will soon be ready for publication.

GENERAL

An interesting pigment producing bacterium, isolated from diseased fish by John Ross, was sent to this laboratory for independent verification of its characteristics. It has the morphology of a Pseudomonas, produces pigment like A. salmonicida, and in physiological characteristics closely resembles A. liquefaciens.

There is great international interest in bacteria of the genus Aeromonas and related types. Cultures from our stock collections were mailed to many laboratories the world over. There was also a voluminous exchange of correspondence and reprints on this subject.

The first commercially manufactured bacteriological culture medium for isolation and identification of fish pathogenic bacterium (A. salmonicida) was marketed by Difco.

A bacterine (bacterial vaccine) prepared from a culture of A. liquefaciens was prepared for immunological experiments to be carried out by R. G. Piper at La Crosse, Wisconsin.

WESTERN FISH DISEASE LABORATORY
Seattle, Washington
Robert R. Rucker, Chief

Seattle Staff
Front row-left to right:--Colleen St.Clair, Nell Nickels, Gail Dryer, Kay Jenes, Sarah Hayduk
Back row-left to right:--W.T. Yasutake, A.J. Ross, Gary White, R.R.Rucker, T.J. Parisot, J. R. Uzmann

The year 1961 brought to completion a protracted laboratory relocation and development plan begun in late 1959. The last of many sub-projects involved, a 40-unit fish holding facility, was ready to use during the last quarter. We are indebted to many for direct and indirect assistance in the completion of these quality facilities.

At the turn of the year the laboratory staff consisted of 10 full-time employees with an accumulative total of 70 years service in fishery biology.

Highlight items of 1961 research activity were as follows:

Techniques were developed for control of Sacramento River chinook disease at Coleman National Fish Hatchery.

Fish tissue culture techniques were perfected and practiced on a routinely successful basis.

Agar diffusion precipitin techniques were successfully developed for typing of fish mycobacterial isolates.

Transovarian passage of mycobacterial disease was disproved experimentally.

Two of four prepared dry pelleted diets were implicated in rainbow trout hepatoma development.

The hypothesis of non-regression of trout hepatoma was substantiated in histopathological findings from a 21-month experimental feeding program.

A second long-term experiment with Hexamita salmonis verified the hypothesis of non-pathogenicity.

Cooperative studies were undertaken with Washington Department of Fisheries to elucidate the management implications of Clinostomum marginatum infections in lake-reared steelhead trout.

BACTERIOLOGY

Studies in serological differentiation of mycobacteria from cold-blooded hosts were continued during the year using diffusion-ingel techniques. Precipitation patterns formed by the immune sera produced to date have yielded from one to six separate immunoprecipitates. Only a limited number of comparative analyses have been conducted as the major emphasis has been concerned with correlated problems of obtaining consistent production of immune sera in reactive mammals. Results to date are promising, however, and indicate that speciation of mycobacteria and other fish pathogens may ultimately be effected by these methods.

Studies were completed on the test hypothesis of transovarian passage of mycobacterial disease in chinook salmon. Spawn from infected and uninfected male and female chinook salmon was cross-paired in all possible combinations, i.e., infected male x infected female, infected male x uninfected female, uninfected male x infected female, and uninfected male x uninfected female. The progeny of these matings were studied bacteriologically and histologically. No evidence was obtained to indicate that mycobacterial disease can be transmitted in or upon either of the gametes.

Identification of the etiologic agent of so-called "red-mouth" disease of rainbow trout was continued. This bacterium does not appear to be allied with any well described group of organisms. Final identification may depend upon serologic studies currently being conducted.

A feeding experiment designed to test the feasibility of pasteurizing salmon viscera infected with acid-fast bacilli was unexpectedly terminated with the sudden loss of experimental fish owing to a water supply failure. While definite conclusions could not be drawn, it appeared likely that the treatment employed was lethal to the mycobacteria present in the viscera. This experiment will be repeated shortly now that new stocks of sac-fry salmon are available.

A motile bacillus resembling both Aeromonas salmonicida and A. liquefaciens was isolated from the intestinal tract of a hatchery-reared silver salmon fingerling. While the organism possessed a single polar flagellum, it also produced a brown-amber soluble pigment on culture media. Biochemical studies indicated close identity with A. liquefaciens. It was concconcluded that the isolate is a unique pigment-producing variant of A. liquefaciens which might readily be confused with A. salmonicida.

PATHOLOGY

Monthly evaluation of the role of dry pelleted diets in rainbow trout hepatoma at Hagerman National Fish Hatchery was continued. Effects of continuous feeding of four different commercial dry feeds have now been studied histologically for 21 consecutive months. Presently, two of the four diets are implicated.

The first of the diet groups which led to hepatomatous manifestations (original suspect diet) was subdivided April of this year into three subgroups to test the possibility of regression or retardation of the hepatomatous trend in the face of dietary change. One subgroup was continued on the original diet, the second was converted to a straight beef liver diet, and the third was placed on a less suspect commercial diet. Histopathological examinations of the livers of these fish seem to substantiate the generally accepted hypothesis that hepatoma does not regress after the initial onset of the disease. Study of these tissues during the last several months seems to indicate an increase in the incidence of neoplasm in all three diet subgroups.

Another item of interest was the observation of a well circumscribed metastasis to the gill of a fish in the 20-month group. To our knowledge this is the first clear-cut gill metastasis seen in fish being held under controlled feeding conditions. Splenic metastatic nodules were also observed in a spleen specimen from the 19-month sample. Frequent bizarre, pleomorphic nuclei and cytoplasmic changes were seen in one of the "non-hepatoma" groups. These livers occasion-

ally exhibited foci of large acidophilic cells with eccentric nuclei similar to those described by Nigrelli and Jakowska (1961). Analysis of future samples of these fish may reveal the significance of these changes.

A second comparative commercial dry diet experiment was initiated at Hagerman National Fish Hatchery by the Branch of Fish Hatcheries. Eighth-month liver samples of fish from the experiment were received during the last quarter. They have been processed and are ready to be examined. Seven different dry feeds are being tested in this experiment. These samples will be the initial materials to be examined. If any atypical histological changes are observed, samples collected in previous months will be analyzed.

1961 trout hepatoma survey materials received from six National fish hatcheries were processed and examined. Reports on the finished materials were prepared and submitted to the central and regional offices.

A dermatitis of yearling rainbow trout commonly called "strawberry disease", was observed in stocks of fish at the Quilcene National Fish Hatchery and other private and State hatcheries. Although often observed by hatcherymen and biologists elsewhere, the disease has not yet been characterized. Bacteriologic and histologic examination of lesions have not revealed any associated etiologic agent. Clinically and pathologically the lesions appear superficial and only "skin deep". However, an unusual number of eosinophiles were noted throughout the circulatory system of one group of the diseased fish. Heavy infection with the blood parasite Cryptobia lynchi was observed in these materials and casts some doubt as to whether the eosinophilic inflammation can be directly associated with the "strawberry disease". Description of the histologic appearance of the disease is being prepared.

Routine diagnostic services were rendered to numerous private hatcheries and Federal and State agencies in Alabama, California, Idaho, Montana, New Mexico, Oregon, Wyoming, and Washington.

PARASITOLOGY

Research in parasitology is primarily concerned with experimental evaluation of chronic parasitic diseases, i.e., those which are persistent, recurring and relatively non-lethal. It is believed that current knowledge of the morphology, life history, and taxonomic aspects of many of the common parasitic disease agents is substantially greater than our understanding of the actual short- and long-term pathologic effects. Of greater moment now is the question of the absolute effects of specific parasites, singly and interacting with others, on the growth response (food utilization efficiency) and stamina or vigor of their hosts.

The effects of chronic parasitism on stamina may be of paramount importance when hatchery-reared fish are ultimately set free in natural environments. It is conceivable that the limiting effects of certain parasitic diseases will not be manifest in direct morbid effects or limitation of growth. Rather, it appears that some forms (i.e., hemoflagellates, myxosporidia) may simply burden the physiologic mechanisms of their hosts to the point of frank physical weakness which will take a large toll of an infected hatchery reared population during the demanding trials of adaptation to the feral state. A working concept of quality control of hatchery reared fishes destined for release into vigorous natural environments must include ways and means of testing the probable success of transition from the state of collective hatchery coddling to competitive free existence.

In line with the objectives discussed above, four parasitic disease agents were investigated during the year.

Evaluation of Hexamita salmonis, asserted pathogen of juvenile salmonids, was completed. A series of experiments initiated in June 1960 were climaxed during the quarter by a 10-week experimental challenge of previously unexposed juvenile silver salmon and steelhead rainbow trout. Test fish and suitable controls were inoculated per rectum with standardized doses of pure-cultured Hexamita or sterile medium. Diet intake and growth response were measured carefully over the 10-week experimental period during

which time all groups of fish exceeded 100 percent gain. Diet levels were held sub-optimal to favor the pathogenic potential of Hexamita. Despite high prevailing levels of Hexamita in all test groups, no significant differences in mortality or growth response were demonstrated between infected fish and non-infected controls. Results of this experiment confirm our previous findings which, briefly stated, are that Hexamita salmonis is not a primary pathogen, but rather, a harmless commensal regardless of levels of infection which might obtain. Anti-hexamita chemotherapy does not appear to be justified.

Chloromyxum wardi, a myxosporidian parasite of the gall bladder and alimentary tract of yearling and under yearling salmonids was found to be widespread in Pacific northwest hatchery trout. Under experimental conditions of imposed starvation, naturally acquired Chloromyxum infections were found to increase in incidence and intensity. Preliminary attempts to maintain Chloromyxum in artificial culture media were partially successful. Studies are continuing.

Clinostomum marginatum, the so-called yellow grub parasite of warmwater fishes, was studied in a unique association with steelhead rainbow trout in a natural environment. Incidence and intensity of infection with Clinostomum larvae exceed greatly the levels reported in any other trout environment. Observed incidence exceeds 90 percent in 2-year outmigrants with intensities of infection ranging up to 17 larvae per gram of biomass. It is tentatively concluded that steelhead rainbow trout are especially vulnerable to Clinostomum invasion and that pond rearing of this strain of rainbow trout may not be feasible in natural bodies of water containing the Clinostomum-Helisoma complex.

The condition characteristics of a series of infected steelhead trout were determined and found to be uncorrelated with the degree of parasitism. These results are in accord with those of Elliot and Rossert (1949) who studied the relationship between condition and infection intensity in yellow perch. We have concluded that the use of the coefficient of condition as a measure of pathogenic effects is invalid and inapplicable in the case of Clinostomum inasmuch as fish infected with more than four Clinostomum larvae per gram of body weight are significantly smaller than those more lightly infected.

Rainbow trout from a commercial hatchery near Auburn, Washington, were found to be infected with the hemoflagellate Cryptobia lynchi. This is a new host record for a species of hemoflagellate known previously from cottid fishes in this State. Experimental infection of steelhead trout and silver salmon has been accomplished. Results of experiments in progress for seven weeks indicated that the disease leads to depression of hematocrit values, pronounced edema and general lassitude. Results of experiment were confounded, however, by the appearance of kidney disease in some of the test fish. This secondary infection is believed to have arisen from the original serum pool which was used as an inoculum. It will be necessary to repeat this experimental program before further conclusions can be drawn.

VIROLOGY

Perhaps the greatest single aid to the study of viruses is successful practice of the methodology of tissue culture. Culture of tissue isolates from primary test animals - salmonid fishes, in our case - greatly enhances the rate of exploitation of fundamental biological facts concerning interaction of the virus parasite and host cell. While other laboratories have had special culture techniques for some time, our efforts in this area have been beset with unexpected technical and mechanical problems. These impediments were overcome and with the concurrent development of a suitable medium we have effected routine culture of various tissues from rainbow and cutthroat trout, silver and chinook salmon. Both embryonic and juvenile tissue sources have been cultured successfully. No attempt has been made to establish cell lines as our present need and interest is concerned with primary cultures.

Over three years of research on the biology of the agent of Sacramento River Chinook Disease (SRCD), a highly virulent virus-like disease of juvenile chinook salmon, has led to development of management techniques for circumventing the disease. Analysis of data from the 1960-61 experimental rearing program indicated that precise management of the

temperature regimen during incubation and early juvenile development could suppress or greatly minimize the disease; briefly, experimental groups of fish which, as eggs, were incubated at 54°F., as sac-fry, at 56°F., and as feeding fry, at 58°F., remained disease free. Application of these and other supporting findings led to development of a recirculation system for water supplies to the hatchery building at Coleman National Fish Hatchery. The major portion of the production fish from the 1961 chinook salmon egg take are currently involved in a full-scale test of this thermoregulated rearing technique.

During the current rearing period we will attempt to pinpoint, in thermal units, the time at which the etiologic agent of SRCD is activated and/or blocked by manipulation of water temperatures. Concurrently, the hypothesis of transovarian passage is being investigated.

Several "virus" diseases were reported by other fishery agencies in this area, but only two were referred to us for investigation. These were infectious pancreatic necrosis (IPN) in rainbow trout and an unspecified malady in cutthroat trout. Both showed vague histopathological changes and general syndromes suggestive of viruses, but laboratory tests were not confirmatory.

The viral hypothesis of hepatoma induction in rainbow trout was not confirmed in our field and laboratory studies. At a September meeting of trout hepatoma investigators, a research team from Colorado A. and M. reported similar negative results in a parallel study and reported plans for continued study using more extensive and elaborate methods. We feel that additional research in our laboratory would be unprofitable duplication of effort and have, accordingly, terminated our investigations.

NUTRITION

EASTERN FISH NUTRITION LABORATORY
Cortland, New York
Arthur M. Phillips, Jr., Chief

Cortland Staff
Front row-left to right:--Henry Booke (State), Donald Livingston
Back row-left to right:--Henry Podoliak, Arthur Phillips, Hugh Poston

The Cortland Laboratory program is a cooperative one with the New York Conservation Department and Cornell University. By long tradition, research results are published annually by the New York Conservation Department in its numbered series "Fisheries Research Bulletin". The Cortland reports may be obtained free upon request either to the Laboratory or the Department.

WESTERN FISH NUTRITION LABORATORY
Cook, Washington
John E. Halver, Chief

Willard Staff
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Back row, left to right:--Warren Shanks, George Cahimer, Charlie Smith, Laurence Ashley, C. Bradford Croston, John E. Halver

Emphasis was continued on the determination of basic nutritional requirements of salmonids and the respective role of each in metabolism. Superimposed upon the basic nutrition program was an intensive study of nutritional factors affecting hepatoma in rainbow trout. Impaired lipid metabolism was emphasized as the most logical primary or secondary precursor for hepatomagenesis.

The threonine requirement of chinook salmon at two water temperatures was completed.

The aminogram technique and extension of indispensable amino acid requirements for salmon, trout and catfish was initiated. Free amino acid content of circulating blood serum of rainbow trout was completed after starvation for three experimental test periods.

The distribution of 18 - 20 amino acids in various proteins commonly used in production diets was assayed on the amino acid analyzer. Limiting amino acid values were obtained for these ingredients and for a number of commercial trout and salmon rations.

The minimum protein requirement for maintenance of salmon and trout was investigated and protein quality measurements plus nitrogen balance studies were improved. Tentative acceptable techniques for the above were established.

The function of steroids in liver tumor regression was emphasized using various nutritional treatments to inhibit or alter hepatomagenesis.

Major nutrient fractions from commercial feeds were studied as vectors for hepatoma and nodules found from feeding the lipid fraction were compared with those induced by 13 classes of chemical carcinogens.

A preliminary survey for lipase activity in salmon caecal enzymes was completed.

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NUTRITION AND BIOCHEMISTRY

Vitamin E requirements of chinook salmon

The vitamin E requirement of chinook salmon were investigated by feeding five levels of the vitamin (0, 10, 20, 40, and 80 mg per 100 gms dry ingredients) in three separate test diets. In one diet, the oil source consisted of highly purified trilinolein included at one percent of the dry ingredients while the fat source for the other two consisted of the mixed triglycerides from herring oil prepared by molecular ...llation. In ... the fat level was ...t one per... ingredients while ... other was ...rcent. In addition, ...rn oil cont... at each of the fat ...els. The ...redients of the diets ...re held co... for dextrin, which was ...red to k... ...ocaloric.

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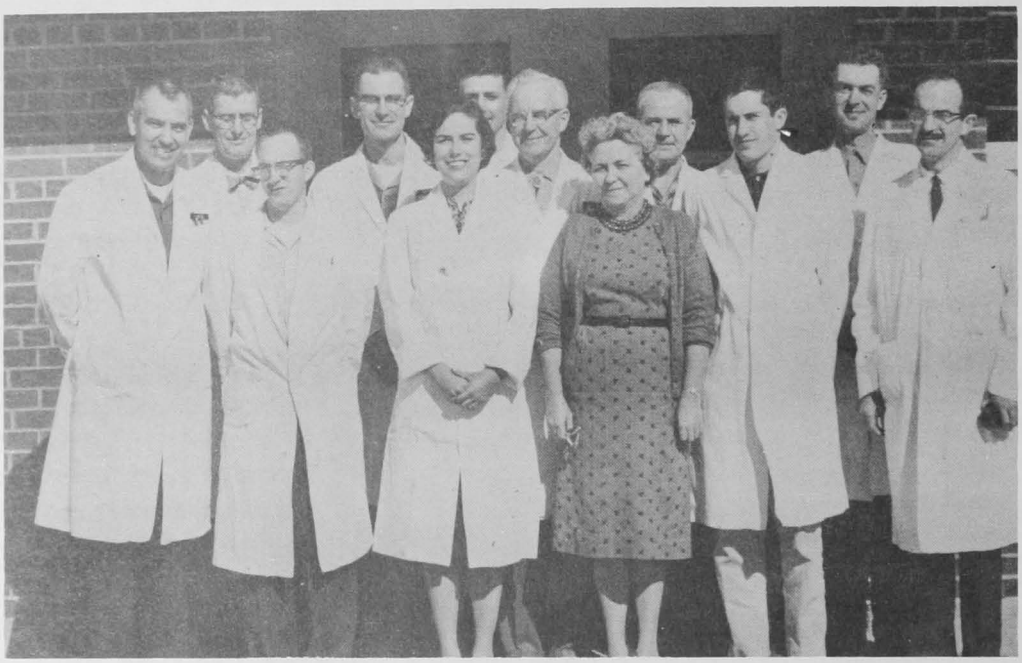
WESTERN FISH NUTRITION LABORATORY
Cook, Washington
John E. Halver, Chief



Willard Staff
Front row, left to right:--E.F. Hesser, David Nash, Helen Paulus, Myrna Morones, Pete Benville, Jr., A.N. Woodall
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Further work on water as a dietary essential was completed and the original concept slightly altered.

An investigation of environment and water chemistry as a factor in white spot disease in chinook salmon fry was initiated. Calcium and zinc balance in the development of eggs and fry were included.

Tissue component changes during extended starvation were continued and a cooperative program with the California-Nevada Sport Fishery Investigations was developed.

The thyroid function in chinook salmon and rainbow trout was investigated using radioactive iodine to thyroidectomize the test animal.

Inter-renal steroids from fish at different phases of migration were investigated.

The normal hematology of chinook and silver salmon was established for major components.

Hemopoiesis was studied histologically together with hematology on the donors.

An intensive study of the histopathology of chemically induced and natural occurring hepatoma was emphasized as well as a more complete study of the dramatic structures and changes in advanced neoplasms.

The library of histopathological material was expanded with approximately 3,000 slides of specific descriptive tissue.

NUTRITION AND BIOCHEMISTRY

Vitamin E requirements of chinook salmon

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Maximum growth response was found with 20 mg in each case. Greatest difference in growth response was noted in the five percent herring oil diets. Fish receiving no vitamin E in the diet were extremely anemic at the termination of the 24-week feeding trial.

Blood cell morphology, histological changes and vitamin E assay on the terminal samples are still to be completed. A weight gain summary is presented graphically in figure 1.

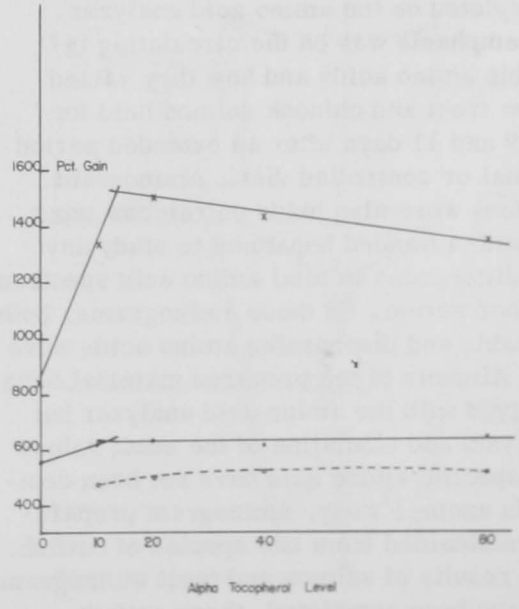

Figure 1:--Vitamin E requirement trials

Threonine requirement of chinook salmon

Analysis of the data for the preceding four years of experimental trials was completed and the finished article submitted to the Journal of Nutrition. Complete analysis supported the conclusions outlined in the 1960 annual report.

Aminogram techniques

The amino acid analyses have been carried out chromatographically on a Beckman/ Spinco Model 120 amino acid analyzer. Preliminary runs have been made on free amino acids in blood plasma and liver tissue extracts to establish specific preparation procedures and quantity of sample to be used. Blood plasma and tissue extracts were deproteinized with picric acid. The resulting precipitate was centrifuged and excess picric acid removed by passing the supernatant through a column of Dowex 2-X10 (200-400 mesh) resin. Next, the effluent of both tissue and plasma was concentrated to approximately 1 ml and made slightly basic for 4 hours to convert cysteine to cystine. Tissue extracts were also treated with sodium sulfide to change glutathione to glutathione-S-sulfonate since glutathione interferes with aspartic acid, threonine, serine, asparagine, glutamine, sarcosine, and proline.

Levels of the free amino acids present in the serum of fish under a starvation regime were completed on the amino acid analyzer. Primary emphasis was on the circulating indispensable amino acids and how they varied in rainbow trout and chinook salmon held for 3, 5, 7, 9 and 11 days after an extended period on a normal or controlled diet. Aminogram preparations were also made on rainbow trout affected with advanced hepatoma to study any possible differences in total amino acid spectrum in this blood serum. In these aminograms, both indispensable and dispensable amino acids were studied. Aliquots of the prepared material have been assayed with the amino acid analyzer but final analysis and tabulation of the exact values for each specific amino acid have not been completed. In another study, aminogram preparations were obtained from two species of catfish. When the results of salmon and trout aminogram studies have been completed, these catfish aminograms will be assayed to predict their tentative indispensable amino acid requirements.

Limiting amino acids in fish feed

Complete amino acid analyses, except tryptophan and cystine, were run on certain commonly employed dietary ingredients as well as on some commercial pelleted preparations. Tryptophan was destroyed during hydrolysis with 6 N HCL. However, tryptophan remains intact by using NaOH as the hydrolyzing agent and a calorimetric method is available for a quantitative determination. Cystine was not calculated because of its low yields and skewed peaks. Data for cystine and tryptophan will be supplied at a later date. The analyses follow:

	ACS	AS	ADF	AM	ACR	AA	AFS	AH	AV	AF	AT	AE
Lysine	8.4*	7.4	7.8	8.4	8.7	8.0	9.0	8.8	8.2	9.1	9.6	9.7
Histidine	2.5	3.3	2.9	3.1	3.7	2.8	2.9	3.4	2.9	2.9	2.4	3.6
Ammonia	5.0	4.7	5.2	5.2	5.1	4.5	4.7	5.4	4.5	4.3	4.3	4.6
Aspartic Acid	8.8	7.9	8.2	8.8	9.0	8.6	8.5	9.4	8.0	8.9	9.3	9.0
Threonine	4.0	3.5	3.7	4.0	4.2	4.5	4.5	4.5	4.3	4.2	4.3	4.8
Serine	4.4	4.0	4.0	4.5	4.5	5.0	4.9	4.2	4.9	4.3	4.6	5.6
Glutamic Acid	14.3	13.9	13.8	14.9	14.9	12.6	12.4	14.0	11.5	13.8	15.0	12.2
Proline	5.1	5.1	4.7	5.0	5.3	5.1	5.1	4.4	5.0	5.2	4.4	5.6
Glycine	5.6	5.7	5.4	4.9	5.4	5.1	5.1	6.5	5.1	8.4	7.4	2.9
Alanine	5.8	5.2	5.4	5.7	5.8	7.2	6.9	6.7	6.6	6.5	6.0	8.0
Valine	5.0	4.4	4.3	4.8	5.2	5.9	5.6	5.1	5.3	4.4	4.4	7.1
Methionine	2.1	1.6	2.0	1.8	2.1	2.5	2.7	2.6	2.4	2.8	2.8	2.6
Isoleucine	3.7	3.1	3.3	3.7	3.7	4.8	4.6	4.1	4.3	3.7	4.0	6.0
Leucine	8.1	6.8	7.0	7.3	8.3	8.2	8.0	7.7	7.9	6.7	7.2	9.7
Tyrosine	3.1	2.6	2.7	3.0	3.3	3.3	3.3	2.7	3.1	2.8	2.9	3.9
Phenylalanine	4.1	3.7	3.6	4.0	4.3	4.3	4.3	4.1	4.1	3.6	3.7	5.1

*Grams amino acid / 100 grams protein

ACS - ACR	Commercial dry diets	AV	Salmon Viscera
AA	Autolyzed mixed salmon products	AF	Salmon Flesh
AFS	Pasteurized mixed salmon products	AT	Turbot (Misc. bottom fish)
AH	California Herring	AE	Salmon Eggs

Minimum protein requirements

Objective of the current group of experiments was to establish minimum levels of nitrogen required for maintenance (it was originally intended to measure endogenous nitrogen in the classical sense, hence the heading for this series). Endogenous nitrogen may be defined as "the minimum essential nitrogen catabolism incident to the maintenance of the vital processes" (Maynard and Loosli). The values reported here cannot be considered true measures of endogenous nitrogen because they were not obtained with animals on a nitrogen-free diet. Further, metabolic nitrogen (again not used in the classical sense) was also included (fig. 2). The values are reasonably accurate estimates of the minimum nitrogen requirements for silver salmon at 52° F. The data shown are for 288-432 hours which was post-absorptive and apparently prior to exhaustion of protein reserve, i.e., while the fish were yet in an equilibrium state.

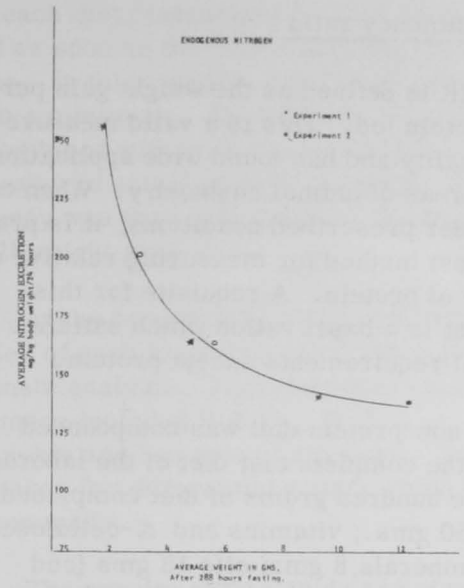

Figure 2:--Endogenous nitrogen values

Silver salmon did not ingest the non-protein diet used previously so an energy source could not be supplied and some tissue nitrogen may have been burned for energy. However, an even greater expenditure of energy would result due to the stress of extra handling during feeding. This was, in fact, evident since nitrogen excretion was higher on days of weighing and sampling where the fish were under some stress. Metabolic nitrogen should be included in the calculations of maintenance nitrogen since "both endogenous and metabolic nitrogen represent fractions which have been actually utilized by the body even though they appear as secretions" (Maynard and Loosli). Tunison et al. (1942) reported that the majority of fecal elimination of fasting fish was in a liquid form. In the absence of any dietary roughage, tissue sloughing is reduced to a minimum so any nitrogen eliminated appears to have been utilized previously.

Results of the first two experiments are listed below:

Average nitrogen excretion- 288- 432 hours at 52°F.

Experiment I

Average weight	9.2	4.8	1.75
Daily nit. exc. (mg/kg)	1.43	166	262

Experiment 2

Average weight	12.3	5.6	3.0
Daily nit. exc. (mg/kg)	140	167	203

Nitrogen balance

Over the past year, the objective of this phase of the research program has been to determine the most rapid and reliable methods for measuring protein quality on a laboratory scale. Protein quality is of major interest to all concerned in this area of animal husbandry since protein probably constitutes the single most important component of salmon diets. Initial efforts were directed toward development of suitable aquaria because recovery of both metabolic wastes and unconsumed food are essential to most nutritional studies. Requirements of the aquaria include rapid drainage, maintenance of uniform environmental conditions and cleanliness. Earlier, aquaria were constructed from glass carboys from which the bottoms were removed. These were efficient but fragile. Ultimately, plexiglass units were fabricated (fig. 3).

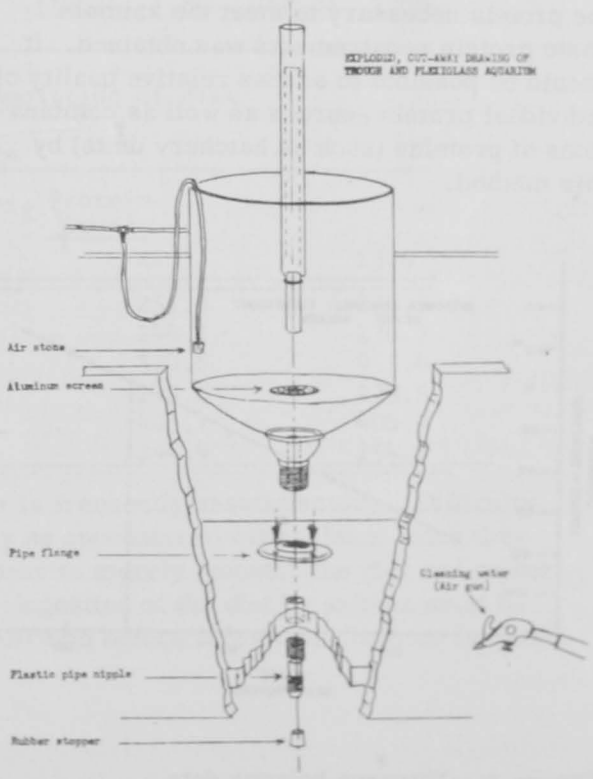

Figure 3:--Experimental plexiglass aquariums

With development of the new experimental aquaria nitrogen balance studies became practical. Of the quantitative measures of protein quality simple nitrogen balance studies appeared to hold the greatest promise. The basic techniques of nitrogen balance studies seemed applicable to the more elegant biological value determinations to be conducted later. Preliminary studies were conducted with the same group of test proteins, i.e., casein, liver and gelatin and non-protein. By the end of 6 days, the gelatin group reached nitrogen equilibrium while casein and liver were in positive balance. The techniques employed were based on Tunison's original studies with trout although several modifications have been made.

Subsequent studies were conducted with casein as the test protein. Experimentation disclosed that levels of 0.5, 1.0 and 1.5 gms of casein diet (25 percent protein dry weight: 12.5 percent moisture) fed to populations of fish of 65 gms bracketed the point of nitrogen equilibrium. Further, the plot of intake versus nitrogen balance proved linear. Nitrogen equilibrium was therefore obtained (fig.4) with 105 mg of casein protein (or 1.6 gms/kg of fish of this size). Thus, a quantitative measure of the protein necessary to meet the animals' basic protein requirements was obtained. It should be possible to assess relative quality of individual protein sources as well as combinations of proteins (such as hatchery diets) by this method.

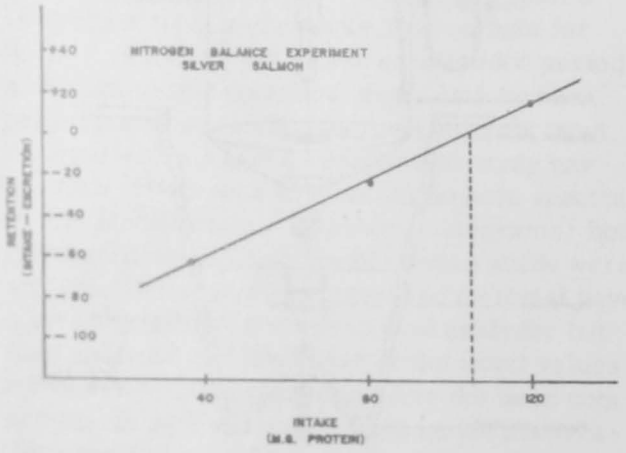

Figure 4:--Nitrogen balance data

Protein efficiency ratio

PER is defined as the weight gain per unit of protein fed. This is a valid measure of protein quality and has found wide application in other areas of animal husbandry. When conducted under prescribed conditions, it is probably the simplest method for measuring relative nutritive value of protein. A requisite for this experiment is a basal ration which satisfies all nutritional requirements except protein.

A non-protein diet was compounded based on the complete test diet of the laboratory. One hundred grams of diet comprised of dextrin, 50 gms.; vitamins and α-cellulose, 18 gms; minerals, 8 gms; oils 18 gms (cod liver oil, 4 gms and corn oil, 14 gms); and CMC, 4 gms. Early in the year a preliminary experiment was conducted using proteins of known quality. Liver, casein and gelatin were tested with a non-protein control. The regular laboratory diet (CTD) was also included for comparative purposes only. The test proteins were fed at a level of 25 percent while CTD is 50 percent protein (casein, 38 gms; gelatin, 12 gms). Results of this 8-week feeding trial with duplicate troughs of rainbow trout were as follows:

PER

Liver 3.09 ± 0.12 non-protein)
) lost weight
Casein 2.74 ± 0.09 gelatin)

CTD 2.13 ± 0.05

The objective of the second experiment was to establish an optimum level of protein for such studies. With other animals, levels ranging from 6 - 12 percent protein are used. Due to the high protein requirement of salmon, higher levels of protein were tested. The diet ingredients to be tested were those commonly employed in hatchery diets and ranged from dry meals to fish tissues. To 50 gms of the basal ration, protein was added to produce levels of approximately 15, 25, 35 and 45 percent. Due to wide variations in moisture and other physical properties exact percentage increments were not calculated. Instead, the ratios of protein component to the basal ration were held uniform at 1.0, 1.67, 2.33 and 3.0 times the basic (15 percent) unit. Dextrin was added to

make each diet "isocaloric". Each diet was halted as soon as the rate of feeding began to decline. In this manner, food wastage was held to a minimum. Kjeldahl analysis of the diet combined with an accurate record of diet fed measured actual protein ingested. Protein efficiency was thus calculated on the basis of overall weight gain.

At the termination of the experiment, samples of each lot of fish were taken for proximate analysis. Hematological examinations made included R.B.C., W.B.C., hemoglobin and hematocrit readings. Smears were taken for differential counts which have not been made.

The results of this study are given below:

Net protein retention

Preliminary NPR studies were conducted with rainbow trout. NPR is a modification of protein efficiency and includes a control group of fish which receive the basal ration (no protein) only. The final weight of this group is subtracted from the final weight of the test group to give net gain. Net gain divided by protein intake gives NPR. The advantages of this procedure include the measurement of protein which does not produce gain in weight and eliminates the effect of intake on protein efficiency.

The first experiments with trout were successful because the trout appeared to ingest the non-protein diet readily. In experiments with other animals ingestion of a non-protein diet is frequently unsatisfactory. Difficulty may be encountered with salmon since they appear to merely "mouth" the diet and reject it. Ingestion of the diet by salmon must be confirmed before further studies are initiated.

Average Protein Efficiency*

Diet	Protein Level							
	X1		X1.67		X2.33		X3.0	
Casein	(17.71)**	2.17	(26.4)	2.11	(38.0)	1.88	(47.8)	2.16
Turbot	(15.6)	2.58	(24.9)	2.34	(30.1)	1.95	(38.2)	1.50
Sal. Meal	(16.7)	2.06	(25.1)	2.59	(34.8)	2.43	(42.1)	2.40
Skim Milk	(13.7)	1.89	(19.6)	2.04	(24.3)	2.15	(28.6)	2.23
Sal. Eggs	(17.8)	2.70	(27.0)	2.82	(33.6)	2.68	(37.1)	2.89
Sal. Flesh	(15.9)	1.98	(25.6)	2.25	(34.2)	1.85	(40.4)	2.00

* PER = Wt. gain/unit protein fed

** Numbers in parenthesis actual protein composition of diet

Average Total Gain

Diet	Protein Level			
	X1	X1.67	X2.33	X3.0
Casein	157.1	213.3	255.3	275.7
Turbot	166.4	239.8	244.1	236.7
Sal. Meal	156.5	294.8	374.6	455.4
Skim Milk	109.8	179.9	249.8	279.5
Sal. Eggs	169.7	326.6	403.1	406.7
Sal. Flesh	101.5	194.9	244.4	254.6

Neither hematological examination nor proximate analysis revealed any quantitative differences. This was not really surprising since these diets were probably all nutritionally adequate, or very nearly so. Maximum protein efficiency was obtained at levels of $15\%_{10}$ and $25\%_{10}$ protein. Also the greatest increment of gain occurred between these levels. Therefore, future studies will be conducted at an intermediate level, probably $20\%_{10}$.

Artificially induced trout hepatoma with classical chemical carcinogens

During the period October 1 to December 31, toxicity effects in high levels of many of the carcinogens-fed groups of rainbow trout forced termination of that particular experiment. In mid-October, all surviving groups of fish at the two highest levels of carcinogen treatment for each specific carcinogen were transferred into outside tanks and the treatments were continued to date. Representative random samples from each of these groups were preserved in alcoholic Bouin's solution for subsequent histopathological analysis. Termination of the following groups was necessary because of toxic effect and excessive mortality experienced in that particular population of trout:

	1/16X	1/4X	1X	4X	16X
2-Acetylaminofluorine				x	x
Aminoazotoluene			x	x	x
Carbontetrachloride					x
DDT		x	x	x	x
p-Dimethylaminoazobenzene				x	x
Diethylstilbestrol	x	x		x	x
Tannic acid					x
Thiourea					x

Experimental dietary ingredients have been accumulated for a confirmatory study of these effects with duplicate lots of initial feeding rainbow trout fry. Only those two levels previously shown in each specific carcinogen to produce an effect will be tested. In those cases where excessive toxicity has previously posed problems, the lowest level and a correspondingly lower level of the carcinogen will be employed. These feeding trials are scheduled to commence January 22 - 25, 1962. The original feeding trials as outlined above will be continued for those lots indicating no hepatoma or adverse effects to date. The minimum dosage of each positive specific carcinogen for trout will be determined by extension of the experimental period for those groups of fish in this category.

Summary of histopathological findings in the liver of rainbow trout fed carcinogens

After 11 months of feeding the specific carcinogens a definite hepatoma was found in the form of early adenocarcinoma of diffuse pattern in aminoazotoluene fed trout at the 1X level. Two different livers showed this picture, one more advanced and with extensive proliferation of cholangioles including several mitoses. All other carcinogens studied produced largely the trabecular pattern of hepatoma. One strongly probable hepatoma nodule appeared after 11 months of feeding the 1X level of DDT. p-Dimethylaminoazobenzene fed fish developed a definite basophilic hepatoma nodule after 11 months at the 1X level. Diethylstilbestrol fed at the 1/16 level developed a hepatoma nodule in one series of samples examined to date.

Dimethylnitrosamine fed rainbow trout developed numerous cases of basophilic staining hepatoma nodules at the 16X level after 11 months. After 14 months the dimethylnitrosamine fed fish developed the most advanced hepatomas observed so far in these experiments. In five selected trout, the livers were greatly enlarged and grossly nodular with multiple hepatoma. Microscopically, these nodules were generally well advanced in intratrabecular necrosis and hemorrhage with varying degrees of fibrosis. Mitoses were present but were not numerous appearing about three per high power field. Considerable admixture of trabecular and adenocarcinomatous patterns were present.

Skin lesions appeared in the thiourea fed fish after 10 months and a broadly erupted skin area developed in approximately 30 percent of the fish fed urethane at the 4X and 16X level for 6 - 8 months. Histopathological analysis of the samples preserved during periodic visits to the Hagerman experimental site have been processed with a 60 - 90 day delay and at the time of this report, analysis has only partially been completed on the October, or 11-month, feeding histological samples.

Suspect commercial ration

Feeding trials utilizing fractions of a suspect commercial ration have been continued. Additional fat, protein, and carbohydrate fractions have been extracted from an additional lot of suspect feed to extend the feeding experiments through January 1962. Surgical inspection and characterization of each fish in each lot fed any level or fraction from the suspect commercial ration was completed in October. Surgical examination and subsequent histological assay of suspect liver areas in fish fed the carbohydrate or protein fractions showed no hepatoma nodules to date. In October, five suspect livers from each of the 6 percent fat and 12 percent fat fed fish were confirmed histologically to be hepatoma nodules.

Extraction of fat from 50 kg of suspect commercial ration has been completed. This extracted fat was fractionated into neutral and phospholipid components by the following procedure:

Aliquots of lipids obtained from the above diet were placed on large chromatographic columns containing silicic acid dispersed in powdered glass. Elution of adsorbed neutral lipid material was performed with extensive washing with diethylether. The phospholipids remaining on the columns were eluted with absolute methanol followed by an elution of 50 percent methanol in water. Approximately 80 percent of the total lipids were recovered as neutral lipid and the remainder as phospholipid. The two fractions thus obtained were concentrated to dryness by several passages through modified circulating and rotary evaporators. During each process, care was taken to maintain a prevailing nitrogen atmosphere with low temperature not exceeding 35° C. After evaporation, the rapid concentrates of both fractions were extracted with diethylether. The neutral lipid fraction showed solubility in this solvent whereas a portion of the phospholipid fraction could not be solubilized without using absolute methanol.

These three lipid fractions obtained from the original extracted suspect commercial ration will be independently tested with small trout and compared with the complete lipid extract, a complete test diet and the commercial ration under study. Feeding trials are scheduled for late January. Eggs have been obtained, fry have been hatched, and initial feeding trials with the complete test diet to teach the fish to feed on this type ration were initiated on December 31. Initial mortality will be removed and representative random samples will be distributed throughout the experimental hatchery during the first two weeks in January.

Factors affecting hepatomagenesis in rainbow trout

The effect of various nutritional treatments on fish with grossly visible hepatoma were again inconclusive on deliberately selected samples of fish in the various prednisolone or cortisone or regression diet fed lots of fish. Examination of the remaining fish was postponed for another three months when a more complete evaluation and termination of these initial studies would be possible.

Partial lobectomies were applicable for the removal of a particular nodule and in those cases, re-examined after partial lobectomy when the complete nodule plus some normal tissue had been removed, no evidence of further hepatoma development was observed in the normal regenerated liver tissue. In those cases when the complete nodule had not been removed, extensive proliferation of hepatomatous tissue could often be seen extending throughout the abdominal cavity.

Tumor transplants in various samples of the recipient blood typed against a four year hepatoma-affected fish indicated some development of the original hepatoma nodule transplanted into the liver, the gonad, the stomach, and muscle. The response varied between individual fish, however, with the transplant apparently growing in only one or two areas, with rejection and normal appearing glandular tissue in many transplanted areas. Within the 10 fish examined, visual evidence of rapidly developing transplanted material was found in the liver, the stomach wall, pyloric caecal area, the testes, and in one case, subcutaneous within the muscle fibrils in the immediately post dorsal area. Subsequent to histopathological analysis of these particular tissues, a new group of transplants will be initiated in January.

Owing to heavy mortalities among the trout injected with hydrocortisone only two samples were obtained for histopathological study. These showed extensive skin and muscle edema which markedly altered the normal architecture of both organs. The trout receiving hydrocortisone did not survive long enough for hepatoma regression to occur.

Trout receiving cortisone acetate survived better than the above but these also died off steadily so that by the end of two months but 5 fish remained. These were preserved and their tissues studied. The livers showed hepatoma still remaining in every case but nodules were present representing early, middle and late stages as well as mixed tumors. Well defined hepatoma nodules usually lacking portal canals were common. No very definite evidence of regression was seen in the tumors but there were suggestions of possible regression in some. This was evidenced by occasional normal mitoses but otherwise little indication of regression was found. Pleomorphism of liver cells and of liver architecture appeared somewhat less in some nodules but since nodules of all stages of development could be seen in these livers, it must be concluded that if cortisone acetate could induce regression of hepatoma it had not been given long enough to bring about the desired results by the time this experiment terminated.

An interesting observation in one of the cortisone acetate injected fish was a rather large zone of pancreatic necrosis between liver and the outer margin of the adjacent lobe of pancreas. The necrotic tissue was surrounded nearly everywhere by a narrow margin of normal pancreas.

Several deposits of ceroid and of melanin were seen in these livers but the significance of these pigments, if any, with respect to hepatoma is unclear.

Malignant hepatomas with metastases

Metastases have been found in at least three adult rainbow trout during the past year. A most unusual case was that of a 17-inch female with hepatomatous metastases to four different organs; namely kidney, pancreas, gill, and stomach. Two others with metastases had tumors in testis only in one case and in kidney and spleen in the other. The incidence of metastases in the hepatomatous trout examined by us, thus far, is approximately 0.5 - 1.0 percent.

Status of experiments

For convenient review, the various feeding experiments are tabulated on the following pages.

Digestive enzymes of salmon caeca

Methods were adapted for the determination of amylase and carboxypeptidase activity in extracts of salmon caeca. These methods showed considerable amounts of both activities indicating important roles in digestion. Both activities appeared in the later stages of our regular chromatography on DEAE cellulose at pH 8.8 (Fraction IV).

An isolation scheme was sought for the preparation of salmotrypsin IV, the endopeptidase we have found markedly different in properties from any known natural trypsin, in more pure and larger quantities than we have obtained by chromatography. It has been found that the pH 4.5 insoluble TAME activity reported by Croston (Archives Biochemistry Biophysics, 89: p.202, 1960) consisted of about 50 percent of the total salmotrypsin IV and no Fraction 1 TAME activity. A procedure incorporating this fact has been developed which yielded moderate quantities of a product having an electrophoretic pattern consisting mostly of one apparently homogeneous peak (fig.5). However, a more pure product is desirable before refined physical-chemical characterization is attempted.

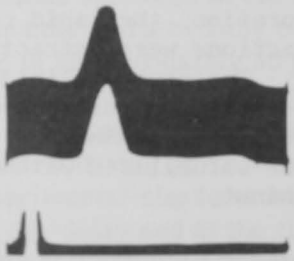

Figure 5:--Electrophoretic pattern of purified salmotrypsin IV. Ascending boundary at pH 6.6

Surgically examined regression groups now at the Hagerman station are:

Surgery Group	Diet	Date of Initial Surgical Examination	Date Terminated
I	70% protein CTD regression	10/25/60	7/12/61
II	Commercial pellet	10/25/60	7/12/61
IIIa	70% protein CTD + hydrocortisone	10/25/60	12/30/60
b	70% protein CTD ± cortisone acetate	10/25/60	1/24/61
IV	50% protein CTD + cortisone acetate	1/26/61	7/12/61
V	50% protein CTD	1/26/61	7/12/61
VI	70% protein CTD	4/8/61	
VII	70% protein CTD + prednisolone[1]	4/8/61	
VIII	70% protein CTD + cortisone	4/8/61	
IX	50% protein CTD with carbohydrate extract	7/17/61	
Xa[2]	70% protein CTD + prednisolone	7/17/61	
b[2]	70% protein CTD	7/17/61	
XIa	50% protein CTD + prednisolone	7/17/61	
b	50% protein CTD	7/17/61	
XIIa[2]	50% protein CTD with 6% extracted fat + prednisolone	7/17/61	
b[2]	50% protein CTD with 6% extracted fat	7/17/61	
XIII	Commercial pellets	7/17/61	
XIVa	Commercial pellets + prednisolone	7/17/61	
b	Commercial pellets	7/17/61	
XV	50% protein CTD with protein extract	7/17/61	
Special Groups:			
	H. Biopsies of hepatoma infected fish	4/8/61	7/14/61
	W. Biopsies of non-infected fish[3]	4/13/61	9/11/61
	H. Tumor transplants	7/17/61	9/12/61
	H. Tumor transplants	7/17/61	9/12/61

[1] Prednisolone consistently administered at 5 mg % dry dietary ingredients

[2] Originally fed diet employing lipid extract and histologically confirmed to have hepatoma

[3] Held at 11° C. at Willard

Diet Group	Induction Diet	Surviving Groups	New Location
63	2-Acetylaminofluorine	1X	121
64		4X	122
67	Aminoazotoluene	1/4X	124
68		1X	125
71	DDT	1/16X	123
77	p-Dimethylaminoazobenzene	1/4X	126
78		1X	127
83	Thiourea	1X	128
84		4X	129
89	Dimethylnitrosamine	4X	130
90		16X	131
98	Tannic Acid	1X	133
99		4X	134
104	Urethane	4X	135
105		16X	136
108	Carbontetrachloride	1X	137
109		4X	138
114	Carbarsone	4X	139
115		16X	140
118	CTD		141

Alkaline phosphatases of salmon blood sera

A method applicable for the determination of the level of alkaline phosphatases in small amounts of salmon blood sera was found by measuring the rate of color development during the hydrolysis of p-nitrophenylphosphate. The method avoids difficulty encountered in using conventional methods which measure inorganic phosphates and give very high blanks for fish blood. Preliminary results are in the table which shows interesting differences in averages for several groups of salmon. However, variations among the limited number of individuals in some groups were great and confirmatory work is needed.

Alkaline phosphatase of salmon sera

Chinook fingerlings on:	
Diet low in Zn and P	27
With added Zn or P	38
With added Zn and P	50
Sea run adults:	
Chinook (12 fish)	55
Silver (11 fish)	101
Spawning chinooks (22):	48
Males (13)	35
Females (9)	66

Species differences in adaptability to dry foods

Results of feeding trials showing the requirements of water in the diet of chinook salmon was reported last year. A paper was prepared for publication but was withheld in order to include supportive data concerning relative moisture uptake and rate of digestion of dry feeds by various salmonid species. Blueback, silver and chinook salmon and rainbow trout were both force fed weighed amounts of dry diet and allowed to feed naturally on the dry diet. Individuals were sacrificed at measured time intervals from 5 minutes to two days after feeding and the moisture and weight of stomach contents determined. The force feeding experiments indicate that rainbow trout pass the food through their stomach at a more rapid rate than do the salmon species. It seemed apparent that none of the species drink after feeding as the increase in moisture of the stomach contents takes place gradually over a 4 to 8 hour period. The natural feeding experiments indicate a difference in feeding habits between salmon and trout. The initial sample always contained a higher moisture content in the trout than in the salmon. As variation between individuals was considerable, additional experiments must be conducted to establish statistically the significance of the results noted. Figure 6 illustrates the rate of moisture uptake following (a) natural and (b) forced feeding.

Calcium, phosphorus and zinc

An extensive feeding trial was conducted in which all combinations of three levels each of calcium, phosphorus and zinc were tested with replicate lots of chinook salmon fry. Although a statistical evaluation of the growth data is not complete, some observations can be reported with confidence. Zinc was shown to be a required element. The addition of zinc sulfate at the rate of 25 mg zinc per kg of dry diet caused a pronounced improvement in growth response in all diets where neither calcium nor phosphorus was limiting. The addition of a second like increment of zinc gave no further growth response, indicating the required level of zinc to be between 13 and 38 ppm (13 ppm zinc is present in the dry basal ration). Although tentative, it would appear that the highest level of calcium fed, 32 gms per kg dry diet, did not exceed the minimum requirement for this element as the maximum gain was noted for this level fed when neither zinc nor phosphorus was limiting. A maximum growth response was also shown for phosphorus at approximately this leve (3.6 gm per kg dry diet) which was in this instance the intermediate level used. Effects noted are illustrated in figures 7 and 8.

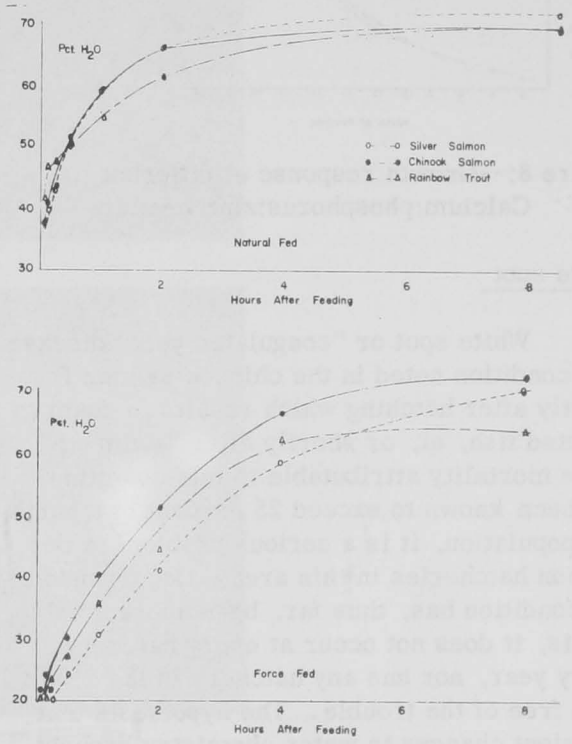

Figure 6:--Moisture uptake into stomach of silver salmon, chinook salmon and rainbow trout after natural feeding and force feeding of dry diet

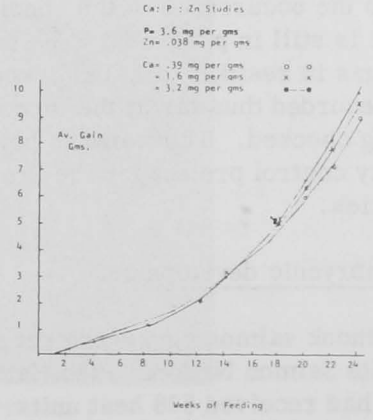

Figure 7:--Growth response at different calcium:phosphorus; zinc studies

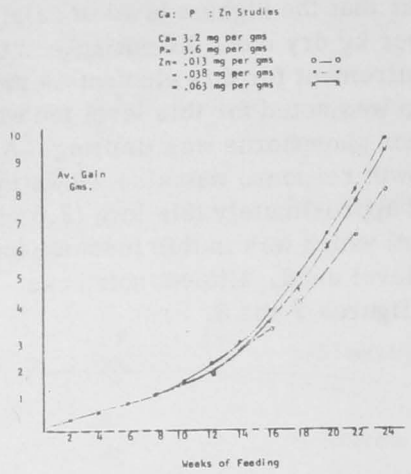

Figure 8:--Growth response at different Calcium:phosphorus:zinc levels

White spot

White spot or "coagulated yolk" disease is a condition noted in the chinook salmon fry shortly after hatching which results in death to affected fish, at, or shortly after "swim-up". Since mortality attributable to this condition has been known to exceed 25 percent of a hatchery population, it is a serious problem to the salmon hatcheries in this area. Occurrence of the condition has, thus far, been unpredictable, that is, it does not occur at every hatchery every year, nor has any hatchery in the area been free of the trouble. The hypothesis that transient changes in water chemistry brought on by weather conditions may be a vector in the condition, is being investigated by making frequent water analyses during the incubation period, to determine if changes observed can be correlated to the occurrence of the condition. The experiment is still in progress. The table shows the changes in residue, pH, CO_2, and zinc analyses recorded thus far at the three hatcheries being checked. This work is being done as a quality control project for the Branch of Fish Hatcheries.

Willard National Fish Hatchery

	9/25	10/20	10/30	11/9	12/5	12/11	12/24
pH	7.37	7.52	7.04	7.45	7.46	7.38	--
CO_2 cc/l	8.53	8.78	10.0	9.13	10.58	9.7	--
Zinc ppm	--	.012	.017	.016	.025	--	.034
Residue ppm	41.2	38.4	38.0	41.4	--	--	--

Little White Salmon National Fish Hatchery

	10/5	10/23	10/30	11/14	12/1	12/11	12/20
pH	7.67	7.32	7.52	7.38	--	7.43	--
CO_2	9.55	8.66	10.4	10.0	10.7	10.0	--
Zinc	.047	.017	.015	--	.010	.034	.015
Residue	43.2	44.2	40.6	44.6	--	--	--

Spring Creek National Fish Hatchery

	10/5	10/23	10/30	11/14	12/1	12/11	12/19	12/20
pH	7.43	7.58	7.64	7.53	7.63	7.42	--	--
CO_2	31.5	32.0	31.5	31.4	31.3	31.6	--	--
Zinc	.015	.014	.010	.012	.024	.010	.051	.029
Residue	114	116	174	102	--	--	--	--

Zinc ion and embryonic development

Eyed chinook salmon eggs were received from Little White Salmon National Fish Hatchery where they had received 528 heat units. Five replicate lots of 1,000 eggs each were placed in separate jar incubators supplied with 500 mls water per jar per minute. Concentration of added ions to the water supplying each group was maintained by metering pumps. The five groups were treated as follows: #1, control; #2, .025 ppm added zinc ion; #3, 0.1 ppm added zinc ion; #4, -0.1 ppm zinc, 20 ppm added calcium; #5, 0.2 ppm zinc, 20 ppm calcium. The following observations are being recorded: egg mortality; hatching rate; fry mortality to feeding age; description of abnormal symptoms observed; change in inorganic composition of eggs and fry at bi-weekly intervals. Effects noted to date are summarized as follows: (1) egg mortalities negligible in all groups; (2) fry mortalities negligible to date; (3) heat units to mean hatch, #1, #4, #5, 900, #2, 920, #3, 940; the hatch of groups 1, 4, and 5 was essentially complete within two days while group 2 was spread over three days and group 3 over four days.

Examination of 10 randomly selected fry from each lot three days after hatching showed that all groups receiving added zinc showed incipient white spot disease (compared with fry three weeks post hatch from Spring Creek National Fish Hatchery diagnosed as characteristic white spot by Harlan Johnson, District Hatchery Biologist). This is illustrated in figure 9; the white spot disease as observed under a dissecting microscope may be characterized as a white opacity around the perimeter of oil globules at or near the surface of the yolk. Larger spots completely surround and engulf the oil globule, and at the very early stages it is not visible with the unaided eye. The addition of zinc and calcium will be continued until the fish are ready to feed, at which time they will be transferred to untreated water in hatchery troughs and fed until the mortalities, if any, have ceased or approached normal.

Figure 10 shows physical science aid Mrs. Hazel Jones, tending the incubators. The metering pumps and controls are in the foreground.

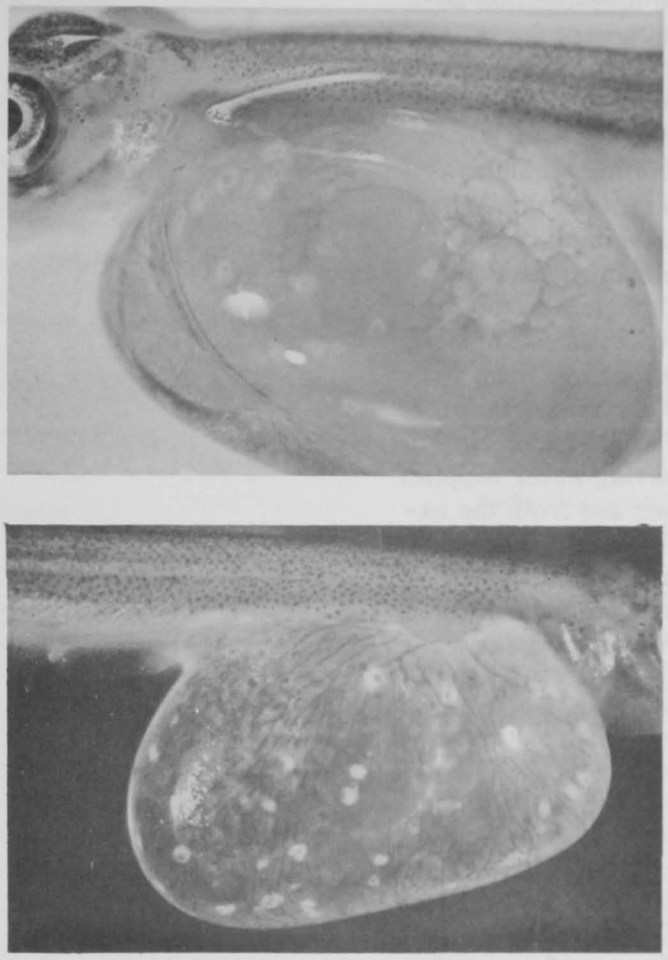

Figure 9:--White spot in chinook salmon fry

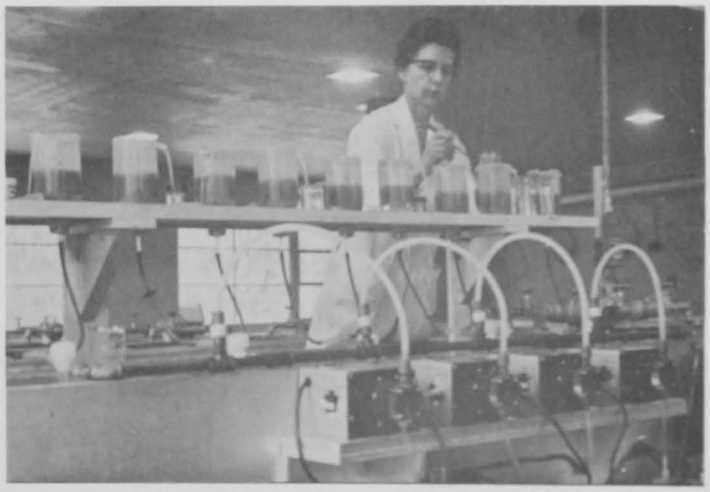

Figure 10:--Experimental white spot aquariums

Water chemical differences versus survival

This is a cooperative investigation between our group, California-Nevada Sport Fishery Investigations, and California Department of Fish and Game with the planning being done together. The Convict Creek staff cares for the fish and is responsible for biological observations and survival data, and the Willar staff assists with some of the sampling and is responsible for chemical determinations and histological observations. In brief, in the pres ent experiment, changes in inorganic composition, blood values, and histology are being followed and compared with survival data for two groups of rainbow trout which had been reared at State hatcheries having water supplies differing both from each other and from the experimental stream at Convict Creek where they were planted. Changes in body composition, in organic composition and blood values are listed in the tables which follow. Survival data and a more complete experimental description will be found in the report of California-Nevada Sport Fishery Investigations.

Convict Creek Experiment 1961

Inorganic Composition

A Stream Holding Pen

Days After Planting	Magnesium		Calcium		Sodium		Potassium		Phosphate	
	MHS	MCr	MHS	MCr	MHS	MCr	MHS	MCr	MHS	MCr
0	1.4	1.4	20.7	12.7	3.0	2.7	13.2	10.6	54.4	36.9
1	1.6	1.9	20.5	18.9	3.6	4.7	15.5	13.5	56.8	55.0
3	2.2	1.9	19.1	21.7	4.1	3.5	16.5	13.7	56.0	52.3
7	1.7	1.5	15.4	20.6	3.8	2.7	18.2	13.5	55.8	52.1
14	2.1	1.6	15.6	19.7	3.5	2.9	18.6	12.4	54.7	46.3
28	1.5	1.4	22.4	23.4	3.0	3.2	11.8	12.8	52.1	52.1
42	1.7	1.8	22.5	22.9	3.2	3.6	11.9	11.0	52.9	51.3
100	1.9	1.2	18.9	26.6	4.4	2.8	13.4	4.7	50.8	51.0
				B Stream Resident						
100	1.8	1.3	18.8	30.8	4.7	3.3	14.4	7.0	51.0	50.2
				C Starvation						
7		2.0		22.6		3.2		14.1		59.2
14		1.7		22.9		2.9		13.4		56.8
28	1.6	1.9	18.7	21.0	2.7	3.4	19.8	13.0	55.2	54.4
42	1.7	1.5	20.5	23.8	2.9	3.6	15.9	11.2	53.7	56.3
100	1.5	1.4	20.9	27.9	4.3	3.3	11.7	7.4	50.5	54.7

Convict Creek Experiment 1961

Body Composition

A, Stream Holding Pen

Days After Start	Moisture		Protein		Fat		Ash	
	Moorehouse Springs	Moccasin Creek	MHS	MC	MHS	MC	MHS	MC
0	71.8	75.6	62.6	67.5	28.8	19.7	9.1	15.1
1	74.6	75.6	66.3	66.6	25.3	24.1	9.3	10.2
3	74.5	76.3	66.4	67.8	25.2	19.1	8.8	11.3
7	73.6	74.2	63.8	65.6	30.3	25.0	7.1	11.4
14	73.5	76.5	63.2	72.1	31.0	17.3	7.1	13.0
28	72.8	76.8	60.2	71.3	30.1	16.9	10.0	13.1
42	73.3	75.0	60.0	72.6	28.6	17.7	10.7	13.4
100	79.3	82.2	78.1	74.0	12.8	6.2	12.2	21.6
			B, Stream Residents					
100	81.0	81.6	82.8	74.9	8.1	5.5	12.0	24.4
			C, Starvation					
7		77.9		69.8		28.8		10.3
14		75.6		68.0		22.5		11.4
28	73.4	78.3	59.1	76.2	33.9	14.6	7.5	12.4
42	74.8	79.1	66.1	75.4	26.7	13.7	9.3	13.8
100	80.2	75.7	77.7	77.7	14.6	8.7	11.7	18.8

Convict Creek Experiment 1961

Hematology

Starvation lot

Days From Start	RBC		Hematocrit		Hemoglobin	
	Moorehouse Springs	Moccasin Creek	Moorehouse Springs	Moccasin Creek	Moorehouse Springs	Moccasin Creek
1	1.28	1.18	40.1	38.1	12.2	12.0
7	1.25	1.42	37.6	43.5	12.2	13.6
14	1.51	1.40	45.4	41.4	14.0	13.3
28	1.34	1.22	45.4	38.4	13.9	12.1
42	1.20	1.23	35.8	30.3	10.7	10.4
100	1.37	.91	33.0	27.5	12.4	9.5
190	.90	--	20.7	--	7.9	--
			Stream Residents			
100	1.50	1.36	39.0	33.5	14.3	11.9
190	1.34	1.38	39.8	37.8	13.1	13.1

Sodium and potassium regulation

To determine if sodium and potassium metabolism is mediated by aldosterone in salmonids, an aldosterone inhibitor, aldactone, is being administered to a group of mature rainbow trout. Serum sodium and potassium levels are being followed by withdrawing a sample of blood via heart puncture at frequent intervals. Changes in endocrine glands will be determined histologically by Dr. M. Olivereau who is collaborating with us in this project. No experimental data can be reported as yet.

HISTOLOGY AND HEMATOLOGY

Hemopoiesis

Impression films of kidneys, spleens, livers, and hearts of spring and fall chinook and sockeye salmon and rainbow trout, totaling 160 slides of six to 12 films each, were made for study of hemopoietic activities. These represent various developmental stages from fingerlings to spawning fall chinooks. Application of this method and study to quality control are anticipated after norms for blood cell development rates for each of the four series of blood cells, reds, lymphocytes, monocytes, and granulocytes, have been established. It is hoped that these studies will provide another diagnostic procedure for anemias and other blood dyscrasias much as is the case with bone marrow smears for humans.

Nutrition histology

Fifteen chinook salmon from the I^{131} thyroid ablation experiments were examined after having become moribund or dead. Smears were made of infected kidneys, spleens or livers which were spotted with 0.5 - 1.0 mm white spots. Stained tissue sections were made of about three of these. Gram positive bacilli were abundant in some of the smears and peculiar spherical giant cells were common in some smears (fig.1). Liver sections frequently showed focal to diffuse necrosis which could hardly have been post mortem change since fish were autopsied and tissues fixed promptly after death.

Hepatoma pathology

Regression:--Five surviving surgically explored rainbow trout all of which had class five hepatomas at laparotomy in November 1960, revealed hepatomas on January 24, 1961. These lesions were histologically confirmed, but they were of altered architecture in the cases of advanced nodules. Early formative nodules were also found. Mitoses were fairly common in some of the advanced tumors indicative of continued growth. An occasional pink nodule, class three, was found and these were taken to represent incipient tumor nodules since they sometimes exhibited one or more of the criteria of hepatoma. Some of the changes seen in these nodules were believed due to cortisone acetate injections.

Induction experiments:--Histopathological findings for samples from 13 carcinogens each fed at five levels, extracted fat fed at two levels, a protein and a carbohydrate fraction, CTD 50 percent, and CTD control have been charted. Most of the above have been followed for 15 months. Class four adenocarcinoma has been definitely identified in aminoazotoluene samples after 11 months, trabecular hepatoma in DDT samples after 11 months, in DAB samples after 11 months, in diethylstilbestrol samples after seven months, in 12 percent extracted fat (incipient) after 12 months, and in 6 percent extracted fat after seven months. DMN samples taken December 11, 1961 showed advanced hemorrhagic, necrotic class four hepatomas--the only far advanced lesions yet seen in the hepatoma induction experiments. These included adenocarcinomatous and trabecular carcinomatous patterns.

General histopathology

Sulmet toxicity and anemia were diagnosed from stained sections of three livers and three spleens of 1961 brood silvers that had been on sulmet therapy seven months at Little White Salmon Fish Hatchery. Samples were collected in November. The hatchery biologist reported very pale gills and almost no blood in the severed caudal artery and vein. Hemoglobin was very low and blood sinusoids in liver and spleen sometimes showed large numbers of rubricytes or young immature red blood cells indicative of the body's

Water chemical differences versus survival

This is a cooperative investigation between our group, California-Nevada Sport Fishery Investigations, and California Department of Fish and Game with the planning being done together. The Convict Creek staff cares for the fish and is responsible for biological observations and survival data, and the Willar staff assists with some of the sampling and is responsible for chemical determinations and histological observations. In brief, in the present experiment, changes in inorganic composition, blood values, and histology are being followed and compared with survival data for two groups of rainbow trout which had been reared at State hatcheries having water supplies differing both from each other and from the experimental stream at Convict Creek where they were planted. Changes in body composition, in organic composition and blood values are listed in the tables which follow. Survival data and a more complete experimental description will be found in the report of California-Nevada Sport Fishery Investigations.

Convict Creek Experiment 1961

Inorganic Composition

Days After Planting	Magnesium		Calcium		Sodium		Potassium		Phosphate	
	MHS	MCr	MHS	MCr	MHS	MCr	MHS	MCr	MHS	MCr
A Stream Holding Pen										
0	1.4	1.4	20.7	12.7	3.0	2.7	13.2	10.6	54.4	36.9
1	1.6	1.9	20.5	18.9	3.6	4.7	15.5	13.5	56.8	55.0
3	2.2	1.9	19.1	21.7	4.1	3.5	16.5	13.7	56.0	52.3
7	1.7	1.5	15.4	20.6	3.8	2.7	18.2	13.5	55.8	52.1
14	2.1	1.6	15.6	19.7	3.5	2.9	18.6	12.4	54.7	46.3
28	1.5	1.4	22.4	23.4	3.0	3.2	11.8	12.8	52.1	52.1
42	1.7	1.8	22.5	22.9	3.2	3.6	11.9	11.0	52.9	51.3
100	1.9	1.2	18.9	26.6	4.4	2.8	13.4	4.7	50.8	51.0
B Stream Resident										
100	1.8	1.3	18.8	30.8	4.7	3.3	14.4	7.0	51.0	50.2
C Starvation										
7		2.0		22.6		3.2		14.1		59.2
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efforts at maintaining a normal red cell population. A corresponding sample taken at the same time from Willard National Fish Hatchery showed normal color of gills and normal blood. Liver and spleen histology were normal.

Forty-five sections were examined from samples of "whirling disease" of fall chinooks taken April 3 at Spring Creek National Fish Hatchery. Histology of head, brain, eyes, gills, digestive tract, heart, liver, and kidney, were all negative. Eighty-five quality control livers received from Eagle Creek, Carson, and Willard hatcheries were sectioned and histopathological diagnoses compared with diets, hatcheries, and species of salmon. Eagle Creek silvers fed Oregon moist pellets showed anemic livers with numerous immature red blood cells (rubricytes). Chinooks from Carson and silvers from Willard each fed on OMP showed only slight congestion in some and mostly normal in other livers. Factors other than, or in addition to, dietary were probably involved in the production of toxic degeneration, congestion, focal necrosis and vacuolation as seen in the subnormal livers examined. These findings are summarized in the following table:

Correlation of liver histopathology with diets of hatchery silver and spring chinook salmon

Hatchery	Diet	Species	Diagnosis
Eagle Creek	Stockton	Silvers	Toxic degeneration
Eagle Creek	Ore. pellet	"	Mild congestion and anemia
Eagle Creek	McNenny	"	Mild toxic degeneration
Eagle Creek	Production	"	Mild toxic degeneration
Eagle Creek	"	Spring chinook	Mostly normal
Eagle Creek	"	Silvers	Mild degeneration and necrosis
Eagle Creek	Control	Spring chinook	Varied from mild toxic degeneration to fairly normal
Eagle Creek	"	Silvers	Fairly normal
Carson	Production	Spring chinook	Mild congestion and vacuoles
Carson	Ore. pellet	"	Slight congestion in one, others mostly normal
Carson	Production	"	Congestion and vacuolation
Willard	Ore. pellet	Silvers	Vacuoles but fairly normal livers
Willard	Production	"	More or less severe vacuolation
Willard	"	"	Fairly normal

Hematology of hepatoma fish

Numerous samples were taken from rainbow trout with known liver tumors. From these blood samples the following tests were run: total erythrocyte count; hematocrit; hemoglobin; and a blood smear was taken for cell morphology. In the early stages of tumor growth an increase in immature cells was noted. Numerous prolymphocytes, rubriblasts and prorubricytes were found in the blood smears. These findings were not always consistent. At the terminal stages of these tumors, an erythropenia was noted to develop with anisocytosis and numerous atypical leukocytes.

Due to the amount of liver destruction caused by these tumors, it was decided to run a series of liver function tests on trout with known tumors, using a fish with a normal appearing liver as a control. An aqueous 5 percent dye solution (bromsulphalein) was used for the injections. The dye was administered on the basis of 2 mg per kg of body weight. This amounted to about 0.2 to 0.3 cc per fish. The fish were anesthetized with MS-222, then placed in a small V board with the gills submerged in a 1:25,000 solution of MS-222. The body wall over the heart was opened and a blood sample taken directly from the heart. The dye was then injected directly into the heart ventrical. Thirty minutes later (after injection), a blood sample was again taken to determine absorption of dye by the liver. This dye was very toxic and if injected by mistake into the heart muscle, and not the ventrical, it caused a fibrilation of the heart muscle and the fish died in a matter of minutes. For this reason the dorsal aorta seemed a more likely point of injection. It was found from the colorimetric readings that the liver absorption of the dye was good in all

but the fish with massive tumors. These tests were all preliminary and considerable work is needed to standardize the present methods used.

Ocean fish hematology

Blood samples were taken during annual ocean collection of silver and chinook salmon. These samples were analyzed for total erythrocyte counts, leukocyte counts, hematocrits, hemoglobins, plus slides for cell morphology. This is the third consecutive year for taking blood samples from ocean caught salmon. The following figures represent the numerical average from the three years:

	Silvers	Chinooks
RBC	1,186,000	1,173,000
WBC	12,800	14,480
Hematocrit	54.0%	49.5%
Hemoglobin	12.0 gm	12.31 gm

Coagulation

A preliminary testing was carried out to determine the prothrombin time in the ocean silver and chinook salmon. A thromboplastin (Owren's method) was prepared from fish brains and was used as one of the extracts for the testing. The other extract was a commercially prepared product simplastin. When performing the test the plasma and thromboplastin-calcium preparation was kept at temperatures equal to that from which the fish was taken. There seemed to be an extension of the length of time required for the bloods to clot in proportion to the rise in temperature. Very few blood samples were found to clot at 37° C.

Fibrinogen

One factor in rapid clotting fish blood was measured clinically (fibrinogen). The method used was described by Gornall, Bardawill, and David. Blood was taken directly from the heart of 10- —14-inch rainbow trout weighing approximately one pound. The two mls of blood taken from the fish was placed in a small vial containing 10 mg of potassium oxalate to prevent coagulation. The blood was centrifuged and care taken to prevent hemolysis. It was found necessary to spin the plasma mixture with the fibrin clot at approximately 5,000 rpm to compress it to the bottom of the tube. On addition of the sodium chloride and biuret reagent, some of the fibrin clots failed to dissolve and some only partially, while some very readily dissolved into solution. The normal range for humans is 200 - 400 mg%. These results were much lower with considerable variation. This was no doubt due to the incomplete resolution of the fibrin into solution. Some modification of the method is necessary before accurate results can be attained.

GENERAL

Quality control

Chemistry:--The specific amino acid distribution in production diets and other dietary materials furnished by the Branch of Fish Hatcheries in this region has been completed. A report on approximately 60 samples which were run through the amino acid analyzer has been tabulated and forwarded to the regional office for use in evaluating the feeding program of the hatcheries in the region. Analyses of a portion of the samples have been reported previously under the section on limiting amino acids in fish feeds. Five samples of dietary ingredients in other preparations for the feeding studies conducted at Abernathy Creek by the Salmon-Cultural Laboratory have been analyzed and the limiting amino acids tabulated. In the materials received and at the reported levels fed to salmon, none of the indispensable amino acids was limiting, although certain amino acid balance relationships appeared important for proper diet formulation. The remaining portion of the chemistry subsection of Quality Control has been the cooperative study between water chemistry and the occurrence of coagulated yolk disease in chinook salmon. This material was reported in the section on white spot.

Hematology:--General hematology has been completed on periodic samples obtained from a number of experimental salmon and trout ponds constructed under the Columbia River program. A summary of the mean values of representative samples of fish from each of these installations has been tabulated and will be forwarded to the regional office for proper use in

evaluating the growth response observed and in the analyses of the experimental fish farm program in the region.

The major effort in the hematology subsection was the organization of a School of Introductory Hematology and Histology for candidates selected by the Branch of Fish Hatcheries within this and other regions. One hundred and twenty hours of instruction and laboratory work outlining the introductory principles of hematology and practical laboratory exercises demonstrating particular techniques was completed during the month of December. Three hours of lecture were scheduled each morning followed by three hours of laboratory demonstrations in the afternoons for five days per week for four weeks. Particular emphasis was placed upon mastery of the current techniques used in routine hematology which might be particularly applicable for use in fish hatcheries or in fishery management. Material covered included common cell morphology, total and differential blood cell counts, hematocrit, hemoglobin, sedimentation rate, and micro-coagulation rate determinations. During the final week of the school a broad general introduction into some of the principles of histology was scheduled with demonstration of the various techniques which would be necessary for proper preservation of material at hatchery or field sites for subsequent histopathological analyses. It was not intended that candidates be instructed in the techniques of histopathology. Proper selection, sampling and preservation of material were covered to minimize receipt of improperly prepared samples when critical histological analyses were expected from the histopathologist. Twenty-three students completed the course and were given certificates itemizing their training.

Construction

Two new laboratories have been completed on the second floor of the recent addition to the main laboratory building and have been equipped respectively for hematology and histopathology studies. Additional heating facilities were installed and completed with the large central heating plant connected to the former small heating system. A diet preparation area has been completed in the new addition to the experimental hatchery in the main laboratory building at Willard and the former cool room has been converted into a 10°F. cold storage room.

At the Hagerman site, additional holding areas for experimental fishes in three section of a small stream have been weired to hold la fish for the hepatoma work. Eight small holdi areas for small lots of larger fish were completed in one large raceway by installing conc block dividing walls and aluminum weirs. A n concrete slab has been poured and a new wate system developed to furnish this area specific designed for more advanced hepatoma feeding experiments with larger fish. Twenty-four 6- foot circular tanks have been installed in this and a prefabricated sheet metal building has b ordered to cover the tank area.

Field studies

An ocean collection trip to obtain sampl from actively feeding adult chinook and silver salmon was completed at Westport during the first week in August. Some of the samples ob tained were: caeca and blood for enzyme stud (Croston); blood for hematology studies (Hess hearts and livers for d-amino acid oxidase an sera for aminogram studies (Halver); sera an samples of many tissues for physiological cha in maturing chinook (LaRoche); pituitaries and other endocrine glands for physiology studies and histochemistry of the primary endocrine organs (Olivereau - from France).

Three intensive experimental surgery in spection trips were completed at the Hagerma station for thorough study of the results of hepatomagenesis in rainbow trout. Over 3,00 individual fish have been surgically inspected with some individuals reopened at least three times. Experimental surgical techniques hav now been perfected to the point where the rout abdominal examination can be completed in fr three to seven minutes, dependent upon the ex tent of liver manipulation.

HUSBANDRY METHODS

FISH FARMING EXPERIMENTAL STATION
Stuttgart, Arkansas
James H. Stevenson, Chief

Stuttgart Staff
Reading from left to right: Fred Meyer, Don Estes, Doris Allen, James Stevenson, William Davis, John Sims (Univ. of Arkansas), David Estes

Construction of the laboratory and auxiliary building at the Stuttgart site was completed and furniture, supplies, and equipment have been installed.

Transfer of the office from facilities in the Game and Fish Building, Little Rock, to the Stuttgart station was accomplished in August.

Extensive progress was made in the construction of the ponds during the fall.

A study of fish farming operations was made over a 4-State area.

An attempt was made to spawn paddlefish under artificial conditions with the use of hormone stimulation. Even though the attempt was unsuccessful, data accumulated indicate that the species will respond to such treatment.

Forty-five chemicals were screened for selective toxicity to green sunfish or tadpoles. Several promising compounds were discovered and are being given further study.

Studies on the incidence and control of diseases and parasites were continued. Myxosporidia were noted to be much more abundant

than had earlier been suspected. Experiments using Mitox to control Lernaea and using Diodoquin and Iodoform for the control of Ichthyophthiriasis were unsuccessful.

Selected reservoirs in the vicinity of the station are being studied to provide data concerning water quality, plankton abundance, and seasonal changes. Soil samples have been taken from all ponds at the station for analysis to provide basic data for future reference.

An excessive abundance of Microcystis was linked to a fish-kill in a pond on a local minnow farm.

Leaflets on fish diseases and parasites; on pond construction; on the history of the Fish Farming Experimental Station; and on the species of fish produced by fish farmers were prepared during the year.

CONSTRUCTION

Construction of the laboratory and auxiliary building at the Stuttgart site was completed in late April. A water supply for these buildings was provided in early August. Pond construction proceeded on schedule during the fall, and the water system to these facilities was installed. Top soil has been added and the ponds have been dressed and finegraded. Crushed rock has been applied to the dikes, roadways, and parking area. However, final inspection of the ponds has not been made, and it will be early 1962 before approval and acceptance can be given.

Several problems arose during the year which affected the development of the station program. Most important was the high mineral content of the water drawn from station wells. Analyses of the water indicate over 400 ppm total hardness, approximately 5 ppm of iron, and traces of sulphur. Specifications are in preparation for construction of a water treatment system. Drainage of run-off water around the station buildings is another problem. During December, water measuring 18 inches in depth flooded the parking areas. Other required changes include modifications for specialized apparatus.

TRANSFER

The Fish Farming Experimental Station was moved from the Game and Fish Building in Little Rock to the Stuttgart facilities on July 24. Furniture and equipment for the offices were installed. Shelving for the library-conference room was erected in November and cataloging of reference materials was begun.

PERSONNEL

The staff at the Fish Farming Experimental Station was expanded during the year. Ray Don Estes, fishery biologist, reported on September 3; David Estes, laborer, on October 1; and Dr. William Davis, physiologist, on December 11. Recruitment of a bacteriologist, a chemist, and a fish culturist is underway but progressing slowly.

FISH FARMING STUDY

A study of fish-farming operations in the southcentral United States was completed. Sixty farmers over a four State area were contacted concerning the nature of their operations. Farms studied varied in size from four acres to 2,010 acres. Farmers were questioned concerning the size of their operations, the physical features of their reservoirs, their water supplies, and the use of the impounded water. Questions were also asked concerning their source of fish for stocking, their stocking ratios, harvest procedures, and approximate yield. An analysis of the data compiled in this survey elucidated several pertinent facts concerning fish farming. There is no standard pattern for construction of a fish farm, various types of levees are utilized, ponds are exceedingly variable in size, and several species of fish may be raised either separately or in combination.

Ponds most commonly constructed on cleared land are built with a bulldozer, using soil from the bottom of the pond. On wooded land a dragline is generally employed to construct the levee using soil from a borrow ditch which may be either inside or outside the reservoir.

The fish farmers were divided into three categories: those raising only food fish, those raising only minnows, and those whose operations utilized both types of fish. In general, the following statements apply:

Approximately 70 percent of the area used exclusively for fish is stocked with food fish. Of the total impounded water studied in this survey, however, over 50 percent of it was in the form of multipurpose reservoirs which were used for such purposes as killing timber prior to clearing, development of hunting areas, for sport fishing, or for storage of irrigation water, as well as for rearing fish.

Depending upon the nature of the operation, ponds vary greatly in size. Producers of minnows or of fingerlings for later sale for stocking may utilize ponds as small as one-half acre. In the production of food fish, on the other hand, reservoirs may approach hundreds of acres. Successful farmers maintain that small reservoirs are much more profitable and provide easier management than large reservoirs, despite the initial increased cost due to construction.

At present three species of fish are most commonly produced. These include the golden shiner (Notemigonus crysoleucas), the bigmouth buffalo (Ictiobus cyprinellus), and the channel catfish (Ictalurus punctatus). Producers of minnows seem to be most successful. Of the food fishes produced, channel catfish appear to be a more profitable crop than buffalo.

Harvesting fish is a costly and time consuming operation. If the water area to be harvested is large, the crop of fish may be so large that some of the fish have died or otherwise dropped in value because of the time and methods used in harvesting.

Parasites and diseases limit production regardless of the species of fish which are produced. Two parasites constitute the major disease problems of fish farmers. These include the "Ich" parasites (Ichthiophthirius multifilis) and the anchor worm (Lernaea sp.). Other parasites of lesser importance but of common occurrence include: Trichodina, Dactylogyrus, Gyrodactylus, and Argulus. Columnaris disease and fungus infections also are important. Invasions by undesirable species of fish or by tadpoles present another problem area.

The control of filamentous algae such as Najas, Chara, and Pithophora, is another major area of concern to the fish farmer. While sodium arsenite is a useful tool, it is only partially effective. Granulated 2, 4-D seems effective in controlling rooted aquatics.

PADDLEFISH

An experiment was conducted on paddlefish (Polyodon spathula) in an effort to ascertain if this species would respond to the use of gonadotropins for stimulation of spawning under artificial conditions.

Seven paddlefish, ranging in size from 8 to 35 pounds, were given injections of 300 units of gonadotropins per pound of body weight. Two days later a second dosage of 200 units was administered. Thirty-two hours after the second injection, 400 units of gonadotropins per unit of body weight were administered. Of the 7 fish, only a 35-pound male responded to the hormones. Necropsies on all of the sacrificed fish indicated the other 6 fish were sexually inactive males and females. Observations on secondary sex characteristics were noted.

SELECTIVE TOXICANTS

One of the most urgent requests from fish farmers concerns the identification of a selective toxicant which would remove green sunfish and/or tadpoles from a pond without affecting desirable species of fish. Forty-five compounds commonly used in agriculture were screened for selective action against either or both of these pests. These experiments were designed only to discover selective action on the part of the compounds, and the LD/O, LD/50 or LD/100 levels were not ascertained. While most of the toxicants were more lethal to the desirable fishes, Diazinon, Guthion, Malathion, Parathion and Trithion show some selectivity to the undesirables without affecting buffalo fish or channel catfish.

Benzene hexachloride, Cryolite, Diazinon, Guthion, Malathion, Maneb, Mitox, Nabam,

Parathion, Phosdrin, Sevin, Sodium Polysulphide, Tedion, and Vapona were only mildly toxic to buffalo fish, and may have usefulness in future tests to control Lernaeid parasites.

DISEASE INVESTIGATIONS

A pronounced rise in the incidence of myxosporidial infections occurred during late August and much of September. Golden shiners were most affected and lesions and cysts occurred in many areas of their bodies. Cysts were identified in the musculature, beneath the scales, in subcutaneous tissues, and on the gill filaments. Acute lesions, however, were detected only on the external surface. Identification of the species involved has not been completed at this time. It appears certain that these organisms fall into the Myxosoma-Myxobolus complex and it is possible that more than one species is involved even though the spores superficially appear similar. An attempt to discover the mode of transmission and to study the course of the disease was made over a 2-month period. Twelve aquaria, each containing 10 liters of well water and given aeration, were stocked as follows: (1) two contained 12 infected fish; (2) seven contained 7 infected fish plus 7 from an uninfected source; (3) two contained 14 fish from an uninfected source which were fed tissues of infected fish; (4) one contained 14 fish from an uninfected source.

In addition, a 10-gallon aquarium was filled with aerated well water and stocked with 25 figerling channel catfish which were then fed macerated tissue from heavily infected shiners. Another aquarium containing 8 gallons of aerated well water was stocked with 20 fingerling carp. These carp were also fed infected tissues.

While the only symptom evidenced by infected fish at the start of the experiment was the presence of numerous cysts beneath the scales and in the musculature, other symptoms became apparent as the experiment progressed. Infected fish exhibited a gradual loss of balance along the horizontal axis and maintained themselves at approximately a 60° angle in the water with an anterior elevation. These fish showed an intense excitability, convulsions, spinning, and, in several cases, scoliosis and lordosis. There was a gradual loss of the originally infected fish with a few succumbing each day. Fish from the uninfected source were alive well after the diseased fish had expired. Approximately 30 days after initiation of the experiment an outbreak of columnaris disease occurred in aquarium numbers 1 through 12. Treatment for this disease was kept at a minimum to prevent possible interference with development of the Myxosporidia.

Examination of the fish which expired during the course of the experiment did not indicate development of myxosporidial infections in the originally uninfected fish. No lesions or developing cysts could be found. The catfish and carp which had been fed tissues containing thousands of cysts also failed to develop any infections. The refractory nature of the fish from the uninfected source cannot be explained at this time. Various workers have reported that only very young fish are susceptible to "myxo" infections. It is entirely possible, therefore, that the "clean" fish may have been too old. Further experiments will be initiated during the 1962 spawning season.

DISEASE VECTORS

A study of the role of undesirable fish as vectors or carriers of disease and parasites was begun. At present three species of fish, the green sunfish (Lepomis cyanellus), the gizzard shad (Dorosoma cepedianum), and the top minnow (Fundulus notatus) have been examined. The limited data collected indicate that the gizzard shad may be important in carrying Trichodinid infections since all specimens of this species examined thus far carried this parasite. The green sunfish harbors species of Gyrodactylus and is infected with Lernaea. Lernaeid infections were also observed in top minnows but it has not been established if the forms collected from the sunfish and top minnows are identical to those attacking golden shiners, buffalo, and carp. Limited experiments in attempting to establish parasites from sunfish and tapeworms on golden shiners were unsuccessful even though the species were kept in close confinement for 30 days. During this period the adult copepods disappeared from the infected fish and it is suspected they picked off the parasites and ingested them.

LERNAEA CONTROL

Mitox, a miticide which had earlier shown little toxicity to buffalo, was applied to Leraeid infected buffalo. In this experiment two aquaria were stocked with 12 infected fish and 12 fish free of parasitism. One aquarium was treated with Mitox at a concentration of 20 ppm, the other was maintained as a control. Daily observations on the aquaria indicated that Mitox at this level had little effect in preventing development of new infections or in eliminating the adult parasite.

ICHTHIOPHTHIRIUS CONTROL

A strain of Ichthyophthirius was established in the laboratory. Three infected channel catfish, each carrying a minimum of 100 encysted parasites, were placed in aquaria containing 10 liters of aerated well water and given feed containing Iodoform or Diodoquin. These compounds were mixed with pulverized pellets of a commercial food used to maintain fish at our station. This mixture was then reformed into pellets with the aid of flour and water. Four diets consisting as follows were applied: (a) reconstituted pellets containing 5 percent Diodoquin; (b) reconstituted pellets containing 5 percent Iodoform; (c) reconstituted pellets with no chemical added; and (d) unpulverized pellets in their original form. All fish were fed at the rate of approximately 1 percent of their body weight per day. Throughout the experiment, all fish refused to feed and eventually died of the disease. To ascertain if the refusal to take food was due to the presence of the therapeutics, some of each mixture was fed to healthy fish of several species. These fish accepted the feeds readily and showed no ill effects. Healthy catfish in another aquarium also accepted the feed without hesitation.

An additional problem thus appears to be involved in the treatment of Ichthyophthiriasis. To date the treatment of this disease has depended upon addition of chemicals to the water. It was hoped that therapeutic agents could be added to feed which would control infections of this protozoan.

OTHER DISEASES

Associated with the fall turnover or the onset of cooler weather, a pronounced rise in the incidence of Columnaris disease occurred. Lernaeid problems ceased to be of significance as the water temperature dropped.

WATER QUALITY STUDIES

Throughout the summer and fall, regular collections of water and plankton were made on 6 selected reservoirs in the vicinity of the experiment station. Two of these utilized well (ground) water and four were filled with surface water. Data collected thus far indicate reservoirs supplied with ground water have a higher reading of total hardness and have higher concentrations of iron. The volumetric determinations of plankton found in reservoirs using surface water are notably higher than those found in reservoirs using well water. Plankton identifications based on these samples indicate that a greater variety of planktonic forms is also present.

In late October a unique situation arose in a reservoir stocked with fathead minnows. This reservoir was surrounded on all sides by similar bodies of water. In the reservoir in question, fish began to die in large numbers. Chemical analyses of the water indicated oxygen depletion was not the cause of death. Examination of specimens in the laboratory indicated that no parasites were present and no lesions were present on the bodies of distressed fish. Further examinations of sick fish indicated a slight dropsical condition and the kidneys of a number of fish were examined. In these, small foci of inflammation were apparent. Attempts to isolate bacteria from these foci on media were unsuccessful. The injection of a suspension of macerated kidney tissue in normal saline solution into fish from an uninfected source failed to produce similar symptoms and it was concluded that bacteria were not the etiological agents. Examinations of the net plankton and bottom fauna from these ponds revealed a striking difference. The net plankton from the suspect pond consisted almost entirely of colonies of Microcystis. The bottom fauna from this pond was also significantly less abundant than that of the surrounding ponds. Water taken from the suspect pond was placed in aquaria.

One aquarium was placed in sunlight, another was given aeration inside the laboratory building. Six minnows were placed in each aquarium and, in both instances, the fish died within four hours. When additional fish were place into these aquaria 12 hours later the mortality was less severe. One-half of the fish survived exposure to the toxic water for a 7-day period. During that time the bloom of Microcystis declined. A subsequent check on the pond indicated that with the decline of the phytoplankton bloom the mortality also slowed. Evidence at hand indicates that the excessive bloom of Microcystis was a factor in the fish kill.

SOIL ANALYSES

Collections of soil samples have been made from the bottoms of ponds at the Fish Farming Experimental Station by cooperating personnel of the University of Arkansas Rice Branch Experiment Station for analyses concerning particle size, organic fertility, nitrogen content and other chemical properties.

Data obtained from these samples will provide a reference for future experiments related to the production of fish and/or rice.

SALMON-CULTURAL LABORATORY
Longview, Washington
Roger E. Burrows, Chief

Longview Staff
From left to right:--E.L. Jacobson, J.H. McCormick, J.W. Elliott, J.L. Shannon, V.R. Whyatt, A.E. Thomas, B.D. Combs, L.G. Fowler, C.W. Casey, R.E. Burrows

The Salmon-Cultural Laboratory was relocated from the Entiat River to Abernathy Creek in March.

Construction of the new facilities was completed in September.

Diet trials on chinook salmon indicated that an increase in the caloric intake by the addition of peanut oil caused a sparing action on the protein.

The stamina tunnel is capable of measuring differences in performance between fingerling chinook salmon reared on different diets and in different environments.

Tests of the recirculating raceway demonstrated that the increase in water velocity produced higher stamina in the fish, greater cleaning efficiency, and better food utilization.

Hormonal treatment of the eggs during water hardening appears to be an effective means of inducing hormone absorption by the embryos.

GENERAL

On March 1 the Salmon-Cultural Laboratory was transferred from the Entiat River in north central Washington to Abernathy Creek in southwestern Washington. All scientific personnel and equipment were moved to the new location. At the time of transfer, remodeling of the utility building into laboratory, library, and offices was in progress. This work was completed during July but facilities were being used within four weeks after transfer. Additional construction consisted of a 1,000 gpm well, two concrete aeration-filtration tanks 20 feet high and 20

feet in diameter for treatment of the well water, a hatchery building 50 feet by 142 feet, and an experimental area consisting of a concrete slab 100 feet by 200 feet complete with water supply and drainage ditch. The completed construction was accepted the latter part of September.

To continue with the experimental program during the construction period, a temporary deck was placed over six of the 8 x 80 raceways and the 42 6-foot circular tanks brought from Entiat installed for the diet trials. In addition, four of the remaining six raceways were converted to the recirculating type for the pond evaluation studies.

Fish-rearing facilities were put in operation in May. A total of 770,000 fingerling fall chinook salmon averaging 760 per pound were received from the Willard National Fish Hatchery. On June 20, 212,000 fingerlings averaging 246 per pound were released in Abernathy Creek. On September 25, 14,000 fingerlings from the diet trials, averaging 35 per pound, were released and on October 17, two experimental groups were released. The first group of 176,000 averaged 18 per pound and was marked by excision of the right ventral fin. The second group of 182,000 fingerlings averaged 29 per pound and was marked by excision of the left ventral fin. A total of 585,000 fingerling fall chinook salmon were released in Abernathy Creek as a result of the combined experimental and production operations.

Fish diseases were a considerable problem in both production and experimental operations during the 1961 season. The cercariae and metacercariae of the digenetic trematode Nanophyetus salmincola were encountered along with the digenetic trematode Sanguinicola, furunculosis, and an unidentified gill amoeba. Of the group, the trematodes present the greatest control problem. Both furunculosis and the gill amoeba respond readily to control measures but serious uncontrollable mortalities developed from the trematode infections.

The freshwater snail Oxytrema silicula is present in large numbers in Abernathy Creek. This snail is the primary host for the larvae of Nanophyetus. During June and July, correlated with rising water temperatures and reductions in stream flow, the cercariae of Nanophyetus entered the hatchery water supply in large numbers. Mortalities as great as 10 percent in a 2-week period have been experienced due primarily to gill damage caused by the invading cercariae. The cercariae have been observed actually to shear off gill lamellae and cause severe hemorrhaging.

The metacercariae were found in practically every tissue of the body with the heaviest concentrations in the gills and kidneys. Delayed mortalities which forced abandonment of the diet trials were attributed to this source.

The role of Sanguinicola in the mortality is more difficult to assess. While it may have been a contributing factor we do not have evidence to indicate it was of major significance.

Control methods for Nanophyetus and possibly Sanguinicola appear to be mandatory if efficient production and experimental programs are to be developed at Abernathy. We are exploring four avenues of approach to this problem. The first is dilution. We will have 1,400 gpm of cercariae-free well water available in 1962 which was not available during the critical period of 1961. With this additional flow we can dilute the creek water with 50 percent of well water during the summer months. This dilution may reduce the incidence of infection to a point that the fish will tolerate.

The second approach consists of the development of a snail migration barrier. During high water periods the snail population is displaced downstream. During low water periods there is a pronounced upstream migration. It is indicated that the upstream population receives recruitment from the lower reaches and, if the upstream migration can be prevented, the upstream population can be materially reduced. As the snail is the primary host in the Nanophyetus cycle a reduction in the snail population should decrease the incidence of the cercariae. Tests indicate that migrating snails will not cross a strip of copper 9 inches wide when the pH of the water is 7.2, but cross readily when the pH is 7.9. As Abernathy Creek has a relatively constant pH of 7.2, a copper barrier appears feasible. We are in the process of installing a

14-inch strip of copper along the crest of Abernathy Falls just below the hatchery water supply intake. We are hopeful this barrier will have a significant effect on reduction of the snail population in the upper reaches of Abernathy Creek.

The third avenue of approach is that of microstraining the creek water supply. Removal of the cercariae from the water supply would, of course, eliminate the problem in hatchery-reared fish. A microstraining apparatus is available commercially which can be equipped with 23, 30, or 60 micron screens. We have subjected the 60-micron screen to a very rigorous test and find that this screen size effectively removes all the cercariae from the filtered water. While this type of device would be adequate for installation in the creek water supply, the equipment is expensive. We feel that other avenues of approach should be explored first.

The fourth and final approach we are considering at present is that of electrocution. An electrically charged grid at the creek intake to destroy the cercariae as they pass between the plates would be entirely practicable providing we can produce lethal voltages economically. We are in the process of determining the voltages and exposure time required to kill the cercariae.

The well water at Abernathy contains 0.9 ppm of ferrous iron and no oxygen when it is delivered at the ground surface. During 1960 a pilot operation was set up to determine the alterations and procedures required to make the well water suitable for fish culture. The filtration and aeration systems developed and tested in the pilot operation were employed in the large aeration-filtration tank prototypes. These tanks have proved very efficient and have reproduced results of the pilot operation in large-scale operations. The aspiration method of aeration brought the oxygen content of the water to 90 percent of saturation and the calcium carbonate filtration beds reduced the iron content from 0.9 to 0.3 ppm. Salmon eggs, fry, and fingerlings have been reared successfully on the well water. Use of the well water will increase both the experimental and production capacity of the station.

We are very pleased with the facilities, versatility, and potentialities of the Abernathy station. While some of the disease problems are serious we do not think them insurmountable. Although the experimental program has been curtailed in 1961 due to relocation, the increased scope of future investigations made possible by the transfer will more than compensate for this reduction.

APPLIED NUTRITION STUDIES

1961 feeding trials

The 1961 feeding trials were designed to explore desirable protein levels, caloric intakes, and vitamin supplementation in a single basic ration. This ration consisted of a composite meal containing 35 percent salmon meal, 30 percent dried skim milk, 20 percent cottonseed meal, and 15 percent wheat germ meal in combination with varying levels of a meat mixture composed of 50 percent each of beef and hog liver. Two protein levels, 20 and 25 percent, were fed. The protein levels were controlled by addition of water to provide bulk in the diet. Three caloric levels, 1,300, 1,650, and 2,000 calories per kilogram of diet were tested. Where it was necessary to supplement the basic ration to attain the desired caloric level, peanut oil was used as the supplement. All caloric levels were calculated on the basis of available calories as developed by Phillips.

Vitamin supplementation was attained in two ways. The first method was by increasing the meat portion of the ration from 10 to 50 percent at 10 percent increments to determine the extent of supplementation required by the meal mixture. Because the calculated vitamin content of certain of the diets indicated a possible vitamin deficiency, a synthetic vitamin supplement adequate to meet minimum vitamin requirements of chinook salmon, as defined by Halver, was added to a duplicate series of trials. Our previous experience with other meal combinations had been so disastrous that we considered the additional vitamin supplementation necessary.

All diets were fed to duplicate tanks of fall chinook salmon fingerlings. The meat-meal

combinations were mixed with water to dilute to the desired protein level, bound by the addition of salt and CMC, and ricer fed. The control diet was the standard meat-vescera-meal combination employed in previous feeding trials at Entiat.

The original intent was to continue the feeding trials for 24 weeks of feeding but due to excessive mortalities the trials were abandoned after 14 weeks of feeding and the data analyzed for the first 12 weeks. Results of these trials are of sufficient interest to warrant a separate detailed report of the experiment. This is in preparation.

A summary of the results of the trials is as follows:

1. The 25-percent protein diets produced significantly greater gains and higher protein deposition than the diets fed at the 20-percent protein level.

2. At the 20-percent protein level, an increase in the caloric intake from 1,300 to 1,650 calories per kilogram resulted in a significant increase in the growth rate and an increase in the protein and fat deposition. A sparing action on the protein by addition of energy calories as peanut oil is indicated.

3. Increasing the caloric level from 1,650 to 2,000 calories per kilogram, while retaining the protein level at 20 percent, did not increase the efficiency of protein utilization or the total gain above that of the 1,650 calorie diets. A protein calorie-energy calorie relationship of 1:1 appears to be near optimum at the 20-percent protein level.

4. Meat supplementation as low as 10 percent was adequate for maintenance of chinook salmon when used in conjunction with the composite meal fed.

5. Vitamin supplementation made no measurable contribution to either growth or survival under the conditions of the experiment. In this experiment the tanks were outside and supplied with creek water. The contribution of natural food available may have been sufficient to fortify a marginal diet.

6. In every instance growth rates and protein deposition in the experimental groups equaled or exceeded those encountered in the Entiat control. The control diet has been tested in production operations and proved very satisfactory.

The 1961 trials will be repeated in 1962 with some indicated changes using cercariae-free well water in inside tanks.

ENVIRONMENTAL FACTORS IN REARING PONDS

The cyclic changes in ammonia and urea excretion previously reported were confirmed in 24-hour sampling of four 8 x 80 raceway ponds. This is the first opportunity we have had to check the large raceways carrying near capacity loads. The patterns of expulsion were similar to those encountered in other pond types.

A report on these findings is in preparation.

MEASUREMENT OF FINGERLING DIFFERENCES

Stamina Tunnel

Tests of the stamina tunnel demonstrate that measurable differences in stamina exist between fish reared on different diets and in different pond environments. Excision of a single ventral fin also causes a measurable reduction in the performance index of the fish. A report on the stamina tunnel as a measurement tool is in preparation.

Reaction measurement device

Time has not permitted complete evaluation of this device. Preliminary experimentation, however, indicates that differences exist within and between different lots of fish in their response to sudden sound. We have not defined all the controllable variables as yet nor attempted to define methods for the measurement of differences.

DEFINITION OF FINGERLING CHARACTERISTICS

The first experiment to define a fingerling characteristic necessary for adult survival was conducted this year. Two groups of fall chinook fingerlings were reared on different diets to produce differences in fat deposition. The Cortland meat-meal combination which is a high-protein, high-calorie diet, was fed to produce a high fat deposition and an Entiat diet which is a low-protein, low-calorie diet, was fed to produce a low fat deposition. Both groups were fed for a 4-month period, marked, and released the latter part of October. At time of release the proximate analyses of the two groups was as follows:

Diet	Water	Protein	Lipid	Ash
Cortland	76.31	16.79	4.98	2.30
Entiat	78.24	16.56	4.18	2.36

Dry Weight

Diet	Protein	Lipid
Cortland	71.60	21.23
Entiat	75.10	17.87

A difference of 19 percent in fat deposition existed between the two groups. Wood, in comparing wild and hatchery fall chinook fingerlings on a dry weight basis reported as follows:

	Protein	Lipid
Hatchery	70.2	19.9
Wild	77.7	15.1

The chinooks on Cortland diet closely approximated the composition of hatchery fish and Entiat diet group that of the wild fish analyzed by Wood. Tests in the stamina tunnel indicated a higher performance index in the Entiat diet fish despite the fact that the Cortland diet fish were significantly larger. Both groups were heavily infested with the metacercariae of Nanophyetus at time of release.

There were 175,000 Cortland diet fish, averaging 18.5 per pound, marked right ventral and 181,000 Entiat diet fish, averaging 29.0 per pound, marked left ventral. Return of adult fish to Abernathy Creek in 1964 will determine if significant differences exist in the survival of the two groups.

IMPROVEMENT OF REARING FACILITIES

Studies of improvements in rearing facilities were limited to further tests of the recirculating and conventional raceways. At Abernathy four of the 8 x 80 raceways were converted to the recirculating type. Comparisons with conventional raceways demonstrated that a 10 percent differential in growth rate in favor of the recirculating type developed after 7 weeks of feeding. This difference occurred when either floating or sinking ricer-fed diets were employed. During the period of heavy infestation with cercariae mortalities were significantly higher in the recirculating ponds. The water-borne cercariae were distributed much more effectively in high-velocity recirculating ponds than in the low-velocity conventional raceways. Tests conducted in the stamina tunnel showed a higher performance index for fish reared in the recirculating ponds than for those reared in conventional raceways.

Similar tests conducted at the Leavenworth National Fish Hatchery with pellet-fed blueback salmon showed no difference in either growth rate or mortality between the two pond types. No difference in disease inhibition was found between the two raceway types. No prophylaxis for control of bacterial gill disease was required and only one therapeutic was given to the heavily stocked pair of raceways on experiment. The pair stocked at poundages comparable to those employed in the Foster-Lucas ponds required no treatment throughout the entire rearing season. This is in sharp contrast to the handling of the Foster-Lucas ponds which require routine, bi-weekly prophylaxis to control bacterial gill disease. Even though the test raceways were not operated at optimum inflows, Mr. Gastineau reports the cleaning time required for the recirculating raceways was one-third that of the conventional type.

These tests conclude our experimentation with this particular alteration of the raceway pond. A report will be prepared showing the recommended alteration and the results of our tests.

SEX CONTROL IN SALMON

The objective in these experiments is to develop methods for the control of sex in artificially propagated salmon. Normal runs of adult salmon contain at least 50 percent males and in some instances as high as 70 to 80 percent males. Hatchery operations require a maximum of 25 percent males for fertilization. At least 25 percent of hatchery escapements, therefore, make no contribution to reproduction of the species. If the sex of hatchery salmon can be regulated to produce 75 percent females the efficiency of artificial propagation can be materially increased.

Our first approach to this problem has been to subject fertilized salmon eggs to various dilutions of the female hormones estrone and stilbesterol during the process of water hardening. In this technique the solution is drawn into the perivetteline space and the eggs exposed to the hormones during at least the early stages of cell division.

The experiment has progressed to the point where we have feeding fish on hand but they are still too small to sex by wet mounts. The results to date indicate that stilbesterol is toxic to both eggs and fry even at the lowest concentration tested, 0.5 ppm. Estrone, however, at levels of 7.0 ppm and below produced survivals in eggs and fry comparable to the controls. At 8.0 ppm and above, higher mortalities were encountered, particularly in the fry stage. We shall continue to rear the experimental lots until sex determinations can be made.

SOUTHEASTERN FISH CULTURAL LABORATORY
Marion, Alabama
Kermit E. Sneed, Chief

Marion Staff
Upper left to right around montage:--Kermit E. Sneed, O. L. Green, Mabel Jones, Harry Dupree, Paul J. Frey (attached to Fish-Pesticide Research Lab., Denver, Colo., working at Southeastern Fish Cultural Lab., Marion, Ala.)

A purified diet composed of casein, dextrin, corn oil, vitamins, minerals, cellulose, and agar was developed for channel catfish. Catfish fingerlings have been maintained on this diet in an apparently healthy condition for five months.

Flathead catfish were spawned and the fry and fingerlings were reared in troughs.

Indirect evidence indicates that failure of channel catfish to spawn in some hatcheries is due to poor nutrition; i.e., insufficient meat or natural food in the diet.

Accelerated development of the ovaries of immature goldfish was achieved by injection of cholesterol pellets containing mammalian chorionic gonadotropins and three extracts of fish pituitaries.

A portable oxygen-temperature meter designed by our laboratory for fish hatchery and pond work was fully tested and found satisfactory.

A portable meter for determination of pH, temperature, conductivity, light, and oxygen was also tested. Publications are being prepared on both of these devices.

Mr. Sneed convened and was chairman of a symposium entitled "The Role of Gonadotropins in the Reproduction of Fishes" for the Tenth Pacific Science Congress in Honolulu.

EFFECT OF THYROID-STIMULATING HORMONE USED ALONE AND COMBINED WITH GONADOTROPIN ON SPAWNING

A paper entitled "The Effect of Thyroid-stimulating Hormone Combined with Gonadotropic Hormones on the Ovulation of Goldfish and Green Sunfish" has been published. Additional research is planned for 1962 on the catfishes and largemouth bass.

EFFECTS OF VARIOUS GONADOTROPIC AND NON-GONADOTROPIC HORMONES USED ALONE AND IN COMBINATION ON SPAWNING

This project will be continued. Past research with fish in apparently "poor" physiological condition precluded any definite conclusions. However, several observations of biological interest were noted and will be pursued.

EFFECT OF VARIOUS HORMONES WHEN USED ALONE AND IN COMBINATION ON PRECOCIOUS OR RETARDED DEVELOPMENT OF IMMATURE FISH

Research has been completed using two mammalian gonadotropins and four fish pituitary fractions. A detailed report of this work was presented in the 1961 quarterly reports and a manuscript is being prepared for publication. Continuation of this work is planned.

SEX REVERSAL AND SEX STERILITY OF FISH

Sex reversal of goldfish by incubating the eggs in estrogens or androgens apparently has failed (detailed discussion presented in 1961 quarterly reports). However, additional work is planned for the spring of 1962 and will involve feeding estrogen or androgen to newly hatched goldfish to the time of sexual differentiation.

THE NUTRITION OF THE CATFISHES

A synthetic diet containing casein, dextrin, corn oil, minerals, vitamins, and cellulose and bound with agar was devised and tested successfully on channel catfish fry (results presented in detail in 1961 quarterly reports). Instructions for preparation of the diet and the results of feeding it to catfish are being prepared for publication.

A satisfactory trough feeding technique for flathead catfish fry and fingerlings has been developed. The results of this work is in manuscript and has been submitted for publication. Research will be continued to find possible methods for reduction of fighting and cannibalism in this species.

We have in progress a project to test three commercial trout feeds and our purified diet. The factors that will be evaluated are rate of growth, food conversion, and mortality. Periodically throughout the test period, samples of fish will be sacrificed so that blood and histological studies may be made. Tentatively, this project will be terminated in October 1962.

GENERAL CHARACTERISTICS OF BLOOD

Studies of blood of the catfishes will be continued in the spring and summer of 1962. Attempts will be made to correlate blood analyses with various nutrition studies. The microhematocrit, ultramicro analyzer, electrophoresis apparatus, and other equipment will be employed where applicable.

BEHAVIOR SOUND OF FISHES

Recorded sounds of fish might be employed to trap or locate fish by playback of spawning, feeding, or aggregating sounds. Also, from a biological point of view, such work may lead to better understanding of the functional and biological significance of various sounds.

We are obtaining equipment and arranging facilities for this work. We hope to begin during the spring spawning season.

A MULTIMETER FOR ELECTRICAL MEASUREMENT OF CONDUCTIVITY, LIGHT, pH, TEMPERATURE, AND OXYGEN IN PONDS, STREAMS, AND LAKES

The instrument has been designed and its components and functions tested. A publication on the device is planned.

An oxygen-temperature meter has been thoroughly tested by our laboratory, Auburn School of Fisheries, and by Dr. Clemens of the Fish Research Center, University of Oklahoma Research Institute. A paper on the instrument is about ready for publication. In addition, a commercial instrument for measurement of pH, O_2, and CO_2 is being tested to determine its value in fishery work.

GENERAL

Largemouth bass were spawned on rubberized hair mats located in a bass brood pond. One nest of eggs was removed to the catfish egg incubator and the eggs hatched successfully. Such a method may allow the fish culturist to hatch fry and stock nursery ponds without the labor involved in moving fry from brood ponds.

We have continued to assist in the teaching program of the Marion In-Service Training School. Currently, each of us is directing a research project for one student. One concerns development of an "air" seine and the other involves physiological effects of rapid temperature changes in fish. Two projects were completed by students during 1961. Ray Hill made tests and wrote a report on use of the oxygen-temperature meter in fish cultural work. Art Olson conducted an experiment on feeding channel catfish broodstock pellets only and pellets with meat supplement. Rate of growth, microhematocrits, and general condition of the fish were reported.

Overwintering of tilapia in troughs in heated buildings is difficult and expensive. However, we overwintered Tilapia nilotica in small earthen ponds which are supplied continuously by water from an artesian well with a constant temperature of 67°F.. We have found that the water temperature in the ponds is in excess of 50°F., even when the air temperature for periods up to one week reached lows of 8°F. - 10°F. Although the average water depth is less than 10 inches, we have been able to maintain in very good condition more than 8,000 pounds per acre of tilapia for periods up to 6 months.

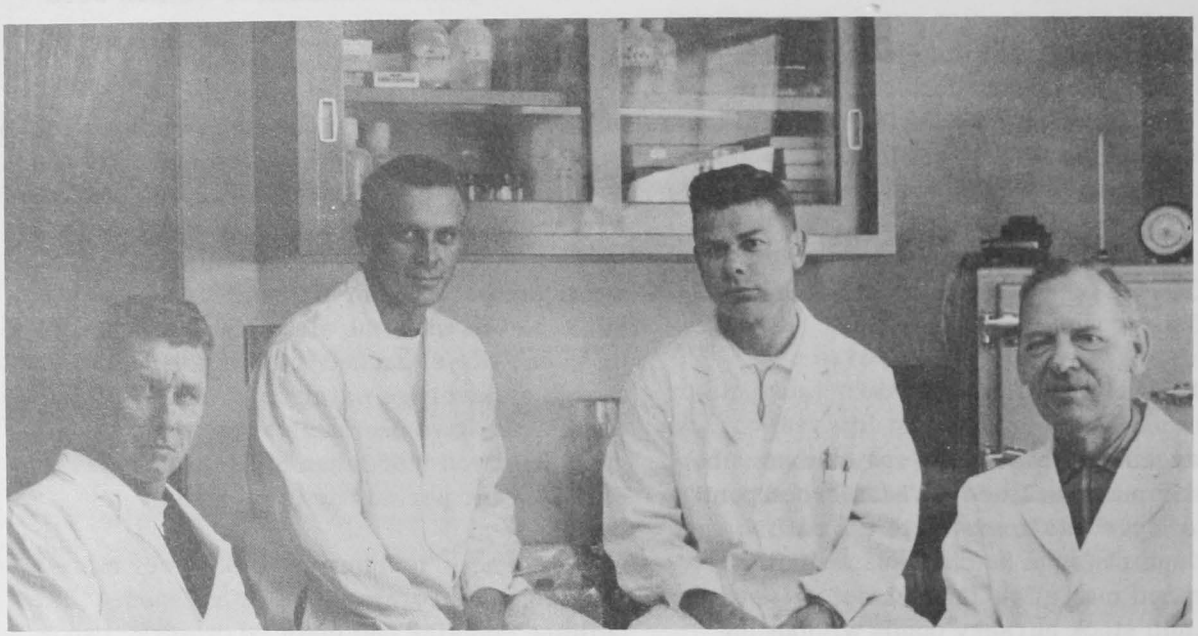
A new "temporary" laboratory more than doubles our laboratory and office space. The building contains one main laboratory, two offices with sink and laboratory cabinets with worktop, one office for Paul Frey of the Denver laboratory, one electrical laboratory, shop and furnace room, and a rest room with shower.

We are also installing a water filtering plant (surplus property) so that our wet laboratory water will be clean and free of food organisms for the purified amino acid experiment to be conducted next summer in cooperation with the Willard Laboratory.

Dr. Dupree attended the course "Principles of Hematology" at the Western Fish Nutrition Laboratory from November 6 through December 1, 1961.

PUBLIC WATER AREAS

CALIFORNIA-NEVADA SPORT FISHERY INVESTIGATIONS
Reno, Nevada
Reed S. Nielson, Chief



Reno - Convict Creek Staff
From left to right:--Norman Reimers, John A. Maciolek, Harry D. Kennedy, Reed S. Nielson

Differences in the stream and starvation survival of rainbow trout reared on pellets with protein levels ranging from 31.0 to 40.0 percent protein indicate that a level of about 35.0 percent is most efficient.

A preliminary experiment demonstrated that transfer of trout reared in a hard water (350 ppm total dissolved solids) hatchery supply to a soft water (77 ppm total dissolved solids) stream habitat produced prompt and severe (26 percent) mortality due to osmotic shock.

A pressure-activated membrane filtration device for field and laboratory use was designed and successfully tested.

Preliminary work was completed on the design and testing of apparatus for the freeze-concentration of solutes in water samples.

The acid dichromate quantitative oxidation technique for analyzing organic matter in limnological samples has been extended by diffusion-titration procedures to include determination of organic carbon and nitrogen as oxidation end products.

SURVIVAL AND VITALITY OF HATCHERY-REARED TROUT REARED ON PRODUCTION AND EXPERIMENTAL DIETS, 1960-61

Stream and starvation tests initiated August 1, 1960, to compare the survival of pellet fed catchable size rainbow trout reared at three California Department of Fish and Game hatcheries (Hot Creek, Fish Springs, and Darrah Springs) were terminated May 1, 1961.

Single lots of trout were received from Hot Creek and Fish Springs and four groups

from Darrah Springs. The groups from Darrah Springs were each fed a different level of protein in the pelleted ration as follows: 40.0, 37.0, 34.0, and 31.0 percent. A high incidence of hepatoma was noted (gross examination) among the Hot Creek (73.0 percent) and Fish Springs (63.0 percent) trout but none among the four diet groups from Darrah Springs. Samples of fish were drawn from each group for hematological, histopathological, and chemical examination by the Western Fish Nutrition Laboratory on May 1, September 1, November 8, 1960, and May 1, 1961 when the tests were terminated.

The experimental stream sections were stocked at a density of 300 pounds per acre and 50 trout from each group were held under starvation conditions. One stream section was stocked with hepatoma infected Hot Creek and Fish Springs trout and one uninfected group (34.0 percent protein) from Darrah Springs to test the possibility of transmission of the disease.

Results of these tests are summarized in the following tables:

Stream survival (percentage)

Hatchery	9/1/60	11/8/60	5/1/61
Hot Creek	94.7	85.5	13.3
Fish Springs	98.0	82.4	14.0
Darrah Springs:			
40.0% Protein		74.0	12.0
37.0% "		82.0	25.0
34.0% "	98.0	86.2	22.0
31.0% "		82.0	11.0

Starvation survival (percentage)

Hatchery	9/1/60	10/1/60	11/8/60	12/31/60	3/14/61
Hot Creek	98.0	86.0	76.0	68.0	46.0
Fish Springs	76.0	50.0	44.0	36.0	24.0
Darrah Springs:					
40.0% Protein	98.0	92.0	74.0	32.0	24.0
37.0% "	96.0	90.0	76.0	58.0	40.0
34.0% "	98.0	98.0	86.0	50.0	20.0
31.0% "	96.0	88.0	76.0	58.0	40.0

Survival was excellent among the fish checked after one month and very good for all groups after 100 days (November 8, 1960) except for the 40.0 percent protein group from Darrah Springs in the stream survival test.

Overwinter (November 8, 1960 to May 1, 1961) survival of all groups, however, was poor especially in view of the unusually mild weather that prevailed. In these summaries it is indicated that the high incidence of hepatoma among the Hot Creek and Fish Springs trout did not result in any unusual mortalities as judged by their survival in comparison with that of the uninfected trout from Darrah Springs. Also, there was no evidence of hepatoma among terminal survivors of the 34.0 percent diet group that were mixed with infected Hot Creek and Fish Springs trout. A comparison of the overwinter survival trends among the four diet groups from Darrah Springs suggests that high (40.0 percent) and low (31.0 percent) protein levels in the diet may be inferior to levels of about 35.0 percent. In an earlier short-term test (May 5 to July 27, 1960) using trout from San Joaquin Hatchery, there was no significant difference in the survival of trout fed 40.0, 37.6, 35.3 percent protein in the diet.

SURVIVAL AND VITALITY OF HATCHERY-REARED TROUT (1961)

In cooperation with the California Department of Fish and Game and the Western Fish Nutrition Laboratory, a preliminary experiment was initiated August 1, 1960 and terminated November 1, 1961, to investigate the significance of the chemical quality of hatchery water supplies in relation to the quality of the trout produced. Moccasin Creek and Moorehouse Springs hatcheries of the California Department of Fish and Game were selected because their water supplies represented the extremes in chemical quality among 11 hatcheries on which analyses are available. Analyses of the water supplies of these stations and of Convict Creek for comparison are presented later in this report.

Operations at the two hatcheries have demonstrated the following differences: more rapid growth of trout at Moorehouse Springs; eggs can be hatched at both but poor success has always been experienced in attempting to rear trout that are hatched at Moorehouse Springs; the feeding of pellets at Moorehouse Springs has usually succeeded in instances where they have failed at other stations; and over-feeding of pellets at this hatchery has never proved harmful to trout as it has at some others.

For this experiment eggs from a summer-spawning strain of rainbow trout maintained at Hot Creek Hatchery were hatched at Moccasin Creek early in September. After one week of feeding, half of the fry were transferred to Moorehouse Springs and the other half maintained at Moccasin Creek. Both groups were fed the same commercial pellet but by mid-December a deficiency was evident among the Moccasin Creek fish and the feeding of another commercial pellet was started and continued for about six weeks before returning to the initial brand of pellet.

The hatchery phase of the experiment was terminated with the arrival of 200 pounds of trout from each station at Convict Creek May 1, 1961. Hauling losses were negligible--six individuals from Moccasin Creek and 12 from Moorehouse Springs. Both lots were placed in holding pens in the stream for observation, sorting, weighing, measuring, and marking. No losses were sustained by the Moccasin Creek trout during this holding period. Mortalities, however, began immediately among the Moorehouse Springs fish with 21 dead on the second day and with all fish exhibiting a pronounced lethargic condition. Mortality increased to a peak of 72 on the fifth day and gradually decreased to none on the twelfth day. Losses for the 12-day holding period amount to 26.0 percent and were attributed to osmotic shock imposed by adjustment from hard water (350 ppm total dissolved solids) at the hatchery to relatively soft water at Convict Creek (77 ppm total dissolved solids).

Fifteen fish from each group were taken for hematological, histopathological, and chemical examination on arrival August 1, and on November 1, by the Western Fish Nutrition Laboratory.

STREAM SURVIVAL

Because of low water conditions, stream survival tests were limited to two experimental stream sections. Moccasin Creek trout were stocked in one at a density of 140 pounds per acre and the Moorehouse Springs trout in the other at a density of 165 pounds per acre. Moorehouse Springs trout selected for this test had an average length of 19.1 centimeters and average weight of 65.7 grams as compared with 13.5 centimeters and 22.1 grams for those from Moccasin Creek. Survival of these trout was checked on August 1 and November 1 with the following results:

Percentage survival

Hatchery	May 1 - August 1
Moorehouse Springs	73.5
Moccasin Creek	32.2
	Aug. 1 - Nov. 1
Moorehouse Springs	49.5
Moccasin Creek	27.5
	May 1 - Nov. 1
Moorehouse Springs	34.9
Moccasin Creek	6.3

These results indicate the definite superiority of the Moorehouse Springs trout to survive under natural stream conditions. However, these trout, although of the same age, were much larger than those from Moccasin Creek and experience in many previous tests has demonstrated size has an important bearing on ability to survive.

At the conclusion of the 184-day test, Moorehouse Springs trout had lost 10 percent in body weight as compared with a 5-percent loss among the Moccasin Creek terminal survivors. These losses indicate a recovery in weight since August 1 when percentage losses were 18.0 and 36.2 for Moorehouse Springs and Moccasin Creek trout, respectively.

STARVATION SURVIVAL

Fifty trout from each hatchery group were placed under starvation conditions May 1 and the experiment was terminated November 1. As shown in the following summary, the Moorehouse Springs trout possessed greater physical stamina than those from Moccasin Creek. All

of the latter trout were dead by September 27, 150 days.

Hatchery	Percentage survival May 1 - Aug. 1
Moorehouse Springs	84.0
Moccasin Creek	52.0
	Aug. 1 - Nov. 1
Moorehouse Springs	28.6
Moccasin Creek	0.0
	May 1 - Nov. 1
Moorehouse Springs	24.0
Moccasin Creek	0.0

HATCHERY-REARED TROUT IN RELATION TO CULTURAL CONDITIONS AND PROCEDURES

Chemical quality of hatchery water supplies

Data on the chemical quality of hatchery water supplies were augmented by addition of analyses from Kern River and Moorehouse Springs Hatcheries of the California Department of Fish and Game. Data are now available on 19 water supplies utilized by 13 hatcheries of this agency. The water supplies of Moorehouse Springs and Moccasin Creek Hatcheries represent the extremes in terms of total dissolved solids and their chemical qualities may be compared in the following summary: (Convict Creek analyses are included for comparison)

(Results in parts per million)

Determination	Moccasin Creek	Moorehouse Springs	Convict Creek
Silica	4.00	32.00	11.00
Iron	.10	.03	.01
Manganese	.00	.00	.01
Calcium	.50	99.00	22.00
Magnesium	.00	5.80	1.00
Sodium	1.00	13.00	1.10
Potassium	.40	2.50	1.70
Boron	.03	.08	.01
Copper	.00	.00	.12
Bicarbonate	9.00	344.00	62.00
Sulfate	.80	9.70	9.10
Chloride	.60	7.00	.10
Fluoride	.20	.10	.01
Nitrate	.40	.10	.12
Nitrite	.005	.00	.003
Nitrogen (NH_3)	.060	.00	.017
Phosphate	.00	12.00	.06
Total dissolved solids	12.00	350.0	77.00
Hardness as $CaCO_3$	1.00	272.00	59.00
Specific Conductance	19.90	536.00	121.00
pH	6.9	7.4	7.9

(Determinations for lithium, strontium, zinc, aluminum, and carbonate were negative on these waters)

PRODUCTIVITY AND TROPHIC RELATIONSHIPS OF ALPINE WATERS

Organic production rates

A preliminary survey of the organic content of four adjacent lakes in the upper Convict Creek drainage has been completed. These lakes lie between 10,100 and 10,700 feet in elevation, contain less than 50 mg/L total dissolved solids, and were selected for analysis primarily because of their contrasting basin conformations. Forty-three water samples taken at different locations and depths were analyzed for total organic content by quantitative oxidation. Analytical values, expressed as organic weight (mg.) or energy (gram calories) per liter, mainly reflect the status of dissolved organic matter which is the preponderant fraction of the total. The results of this survey may be summarized as follows:

1. Generally, low organic levels prevailed in these alpine waters but each lake appeared to have a characteristic organic content. Average organic values for them ranged from 1.1 to 1.7 mg./L., (or 5.4 to 8.1 gcal./L.).

2. Between-lake differences apparently were not related directly to water temperature, total mineral content, or basin morphometry. The lowest values, 1.05 - 1.14 mg. organic matter per liter, obtained from a lake whose sulfate content exceeds bicarbonate, imply that ionic balance of waters may exert a strong influence on organic content.

3. Vertical variation in organic content was noted only in the deepest lake (maximum depth 290 feet) which was manifested as a minimum value at an intermediate depth.

4. Within-lake temperature effects were observed in a shallow lake having four distinct arms. The organic contents (1.22 to 1.84 mg./L.) of subsurface water varied directly with temperature (44 to 49°F.).

These survey results suggest that the organic levels of alpine lakes may bear a realtionship to their productive abilities; and, that total organic content is a readily measurable parameter by which the effects of environmental influences on productivity might be evaluated.

These aspects of the problem will be investigated in a broader survey of the total dissolved and particulate organic contents of lakes in the Sierra Nevada region.

Production of periphyton in lakes

The production of periphyton in Convict Lake during the fall overturn was evaluated by conventional gravimetric methods and by quantitative oxidation. While results of the two analytical techniques showed good agreement, the oxidative method demonstrated superiority in rapidity and sensitivity of analysis.

The periphyton population was observed to be almost exclusively autotrophic, and its environmental growth conditions such that temperature and nutrient supply were homogeneous throughout the watermass. Quantitative analyses indicated that under these conditions periphyton growth was limited by light intensity -- the depth distribution of the organic mass being analogous to a logarithmic light penetration curve. Significant increases in periphyton production near the lake bottom in shallow areas were attributed to reflective increases in light intensity.

These observations suggest that lake basin morphometry exerts a strong influence on autotrophic production at fixed points within the watermass when light is a limiting factor. This morphometry-production relationship will be studied more thoroughly by the peryphyton technique with analyses facilitated by quantitative oxidation.

The field portion of a study designed to detect spatial variations in light - limited primary production of a mountain lake has been completed. Periphyton sets were established at 16 stations in 8-acre Laurel Lake at the onset of fall overturn. These sets were recovered after a 22-day growth period which was characterized by a mean water temperature of 40.1° F. Data collected represent simultaneous algal growth accumulations from 42 separate locations within the watermass. Results of qualitative and quantitative analyses of this material will be variously compared to demonstrate the spatial productivity pattern which may be further related to basin conformation and orientation.

METHODS AND APPARATUS FOR LIMNOLOGICAL STUDIES

Membrane filtration device

The design and testing of a simple, durable, pressure-activated membrane filtration device for field use was completed. The unit consists of a 500 ml polyethylene bottle with a bicycle tire valve mounted in the base and with the bottle cap designed to hold a stainless steel funnel and washers to secure 3/4-inch diameter filter discs. Air is pumped into the bottle through the valve by means of a small hand pump. The apparatus performs rapidly and efficiently.

Freeze-concentration of solutes in water samples

The dilute nature of solutes and suspensoids in alpine water poses analytical problems, especially in determinations of heat-labile organic matter. A technique which involves the slow freezing of such samples as a means of concentrating solutes, was designed and tested. The method involves the treatment of four 1-liter samples in capped polyethylene bottles with the bottle necks protruding into an insulated box. The interior of the box is warmed by the heat of a low-wattage light bulb and the entire apparatus with samples placed in a freezer. Proper temperature regulation allows clear ice to form slowly excluding solutes which become concentrated in the unfrozen water in the necks and centers of the bottles. Preliminary trials have yielded liquid concentrates of about one-eighth of original volumes that contained over 90 percent of the total dry solids.

PAPERS IN PREPARATION

Maciolek, J. A.
A simple pressure-filtration flask.

Reimers, Norman
Water temperature fluctuation as a factor in late winter mortality of hatchery trout in Convict Creek.

MARINE GAME FISH RESEARCH CENTERS
Sandy Hook Marine Laboratory
Highlands, New Jersey
Lionel A. Walford, Chief

Sandy Hook Staff
Seated - left to right:--June Krayl, Elizabeth Postle, Roberta Carter
Standing - left to right:--R. Eisler, Robert Wicklund, W.P. Jensen, L.A. Walford, J.R. Clark, J. Prager, L. Maxie, J.G. Casey

NATIONAL STATISTICS ON SALT-WATER ANGLING

As an adjunct to the Bureau's 1960 National Survey of Hunting and Fishing, the Sandy Hook laboratory undertook a special statistical evaluation of salt-water angling. We contracted with the U. S. Bureau of the Census to collect the basic data by an area probability sample. The sample consisted of approximately 18,000 households containing 45,000 persons. John Clark is analyzing the data and preparing a report for publication. The results indicate that over 6 million salt-water anglers caught close to 633 million fish during 1960, and that the average annual catch-per fisherman was 102 fish. The groups of species most frequently taken were: weakfishes (80,200,000); croakers (41,700,000); and flatfishes (21,800,000).

The statistics break down as follows:

By fishing methods	Millions of fish caught
Still-fishing from boats	292
Casting and trolling from boats	184
Still-fishing from shore	85
Casting from shore	72
total	633

By fishing regions	Millions of fishermen	Millions of fish caught
North Atlantic	1.2	97
Middle Atlantic	1.3	115
South Atlantic	1.0	157
Gulf of Mexico	1.4	185
Pacific Coast		
Southern Calif.	.7	50
Northern Calif. to Alaska	.7	29
Total	6.3	633

RESEARCH ON BASIC FOOD ORGANISMS

To enlarge understanding of the principles controlling the production of food of game species, we have undertaken a program of research on the microscopic plants and animals which are at the bottom of the food pyramid. The microbiological laboratory, starting from three dilapidated basements rooms, has become an attractive, well equipped, operating reality. A large preparation room is equipped for synthesis of chemically defined artificial sea-water media, isolation and inoculation of cultures, and microbiological assay procedures. The service room connecting with it contains facilities for steam and dry-heat sterilization, incubation of bacterial and protozoan cultures above room temperature, and preparation of chemically clean glassware. The constant temperature room houses a 9-bank light source fronted by sufficient shelves for thousands of test tube cultures or hundreds of flask cultures. Space is reserved for a research microscope as well as additional light banks and working area. Dr. Prager has isolated 30 species of diatoms and dinoflagellates from Sandy Hook Bay and is now growing them in chemically defined media in preparation to establishing them in axenic cultures (i.e., in absence of other species). These cultures and those donated by Dr. John J. A. McLaughlin of Haskins Laboratories, Inc., New York, are for use in experiments on biochemical interrelations among phytoplankton microorganisms and in nutritional and physiological studies on individual species.

EXPERIMENTAL FISH CULTURE

The aquarium for experimental culture of marine fishes, having a total capacity of about 5,000 gallons, was completed late in the fall. Water is pumped in at a rate of 23 gallons a minute from a well sunk 57 feet underground, located just back of the breakwater. Ronald Eisler, who joined the staff during construction of the system, has determined that during the first three months of operations the temperature, oxygen content, pH and salinity have remained constant, i.e., 13.5°C., 6.0 ppm, 7.6 and 23 percent, respectively. Turbidity, originating from a clay used in the well construction, is gradually diminishing. Fish of several species have adapted to the aquaria, including winter flounder, eel, blackfish and mullet.

Winter spawners, i.e., fluke and winter flounders, are the first subjects for experimental studies of development and survival of young under different environmental conditions. These studies are in progress.

COLLECTED PAPERS ON SEA-WATER SYSTEMS

The laboratory is sponsoring publication of collected papers on design, construction, and maintenance of sea-water supply systems for experimental aquariums. This project developed as an outgrowth of the design of sea-water experimental facilities for this laboratory. We found little published information to guide us and found that many others had suffered from the same lack in designing systems for their laboratories.

For this publication we have asked contributors to describe their experiences in the design, construction, and maintenance of sea-water supply systems for experimental facilities, emphasizing unique features employed to combat problems such as fouling, siltation, equipment breakdown, and toxicity of components.

Among prospective contributors contacted in North America, Europe, and Asia, 20 have agreed to send contributions which Mr. Clark will edit at Sandy Hook and submit as one volume in the Bureau's Research Report series.

PLANKTON RESEARCH

In order to maintain a year-round record of relative abundance and species composition of eggs and larvae of fishes occurring in Sandy Hook Bay, and to collect material for comparison with

the biotic regimes in other estuaries, we established a program of systematic sampling beginning in the late fall of 1960-61.

Robert Croker or a student assistant has taken a plankton sample once weekly from a stationary boom built for the purpose on the base of one of the pilings of Highlands Bridge. During summer and fall they also took periodic samples at five other points from the sea to the upper reaches of the Navesink River. In addition we arranged with the Coast Guard to take periodic plankton samples at Scotland Lightship. The collections have been sorted and the fish eggs counted, identified and tabulated.

SHARK STUDY

From August 13 to October 13 the laboratory undertook a systematic study of distribution, abundance, species composition and food habits of sharks, and also a hydrographic survey in coastal waters from southern New York to northern Delaware. The project was under the general direction of John Clark. It was made possible by the generosity and cooperation of a number of organizations. The Smith Research and Development Corporation loaned the research vessel Cape May and contributed its operating expenses. The New Jersey Department of Conservation and Economic Development and the New Jersey Resort Owners Association contributed funds and personnel. Other cooperating institutions were the Pascagoula Exploratory Fishing and Gear Base and the Beaufort Biological Laboratory of the Bureau of Commercial Fisheries, the New Jersey Marine Fisheries Laboratory, New York Zoological Society, Delaware Game and Fish Commission, Lamont Geological Observatory, U. S. National Museum, and the Woods Hole Oceanographic Institution.

We made repeated oceanographic observations along eight standard transects extending from shore 40 miles seaward. Whenever possible we occupied fishing stations at 10-mile intervals along these transects; and also made a special cruise in the Hudson Canyon during early October. Using Japanese longlines, bottom chain gear and gill nets we caught 10 species of sharks (311 specimens) on 41 fishing sets. Large sharks included the tiger, mako, great white, sandbar, dusky, sand, thresher, and two species of hammerheads. The largest specimen was a 12-foot tiger shark weighing 1,100 pounds; the species most frequently taken was the sandbar shark. We collected six species of teleosts, namely, swordfish, white marlin, dolphin, yellowfin and bluefin tunas, and albacore. Results of the shark study indicate that: (1) large specimens of the sharks caught were common off the New Jersey coast during the period of the survey; (2) they are unevenly distributed; (3) specimens which we caught had eaten a variety of foods, among which garbage and fish cuttings figured prominently; and (4) although we caught large sharks throughout the survey, the numbers decreased when the surface temperature dropped below 66°F. The survey aroused an astonishing amount of public interest, as reflected by the large number of letters requesting information on shark fishing. It appears likely that as a result of this investigation a concerted effort to fish sharks for sport will be made next summer. John Casey is analyzing the data and preparing reports for publication.

DISTRIBUTION OF GAME FISHES

We have been assembling and tabulating historical data bearing on annual and seasonal changes in availability and distribution of several species of game fishes, including bluefish, weakfish, croaker, mackerel, scup, striped bass and white perch. It appears that each of these fishes has a particular range of temperature tolerance, the distribution and movements of local populations being determined accordingly. In spite of overlapping ranges, differences in optional temperatures are sufficiently distinct to explain differences in distribution and in responses to environmental changes. This study will be the subject of a report by Lionel Walford.

STUDY OF SURFACE TEMPERATURE REGIMEN

George West is continuing analysis of surface temperatures in the western north Atlantic during 1955 and 1956, using data provided under a cooperative arrangement by the Washington Laboratory of the Bureau of Commercial Fisheries. He has completed 78 charts, each representing averages for 5-day periods, in preparation for study with comparable meteorological records.

ENVIRONMENTAL ANALYSIS - ATLANTIC SHELF CAMPAIGN

A question of great interest to anglers and conservationists is this: What is responsible for the ups and downs in fishing luck, for variations in the places and times that fish appear near the coast, in the duration of their stay, and in their abundance? This question will never be answered without thorough environmental analysis; i.e., biological and physical oceanographic studies of the water in which the fish live. That means the zone of the continental shelf (from the inner reaches of estuaries out to the steep slope beyond the 100-fathom curve) where over 95 percent of the Atlantic game species spend their entire lives. Environmental analysis can be fruitful for bringing out large-scale pictures only if based on data collected simultaneously and at frequent intervals over long periods. It requires collaboration among all kinds of scientists--ichthyologists, general zoologists, botanists, physical oceanographers, chemists, geologists and geographers. Although it is beyond the capacity in equipment, manpower and funds of any one institution, people engaged in practically all the disciplines of the marine sciences need the information required for environmental analysis. They may need it for different purposes but they do need it; and are frustrated from pursuing many lines of research because it is lacking.

The Sandy Hook laboratory has proposed a plan of inter-disciplinary, inter-institutional cooperation in planning and conducting a systematic and synoptic survey of the Atlantic continental shelf to begin in 1965. Presentation of the idea to the Atlantic States Marine Fisheries Commission and the Shallow Water Conference led to organization of a Committee of marine scientists representing agencies of Federal and State governments, universities and private research laboratories. The conception of the program, which is tentatively called the Atlantic Shelf Campaign, is accepted and the Committee of which Lionel Walford is the chairman, has begun its task of planning.

DEDICATION

The Atlantic Marine Game Fish Research Center was formally dedicated on September 28, with ceremonies attended by about 400 distinguished guests. Henry Lyman, publisher of the Salt Water Sportsman, introduced the principal speakers, who were Secretary of the Interior Stewart Udall, Senator Harrison Williams of New Jersey, and Fairfield Osborn, President of the Conservation Foundation. An afternoon open house following the program featured exhibits and demonstrations relating to laboratory activities. An evening buffet featured sea food from each of the Middle Atlantic States. A number of organizations and individuals expressed their good wishes for the success of the marine game fish program by contributing in various ways to assure the success of the occasion. We are particularly grateful to the Atlantic Coast Marine Sportsmen's Association, The New Jersey Resort Association, The Salt Water Sportsman, the Sport Fishing Institute, The Garcia Corporation, Field and Stream, the Long Island Shellfish Farmers Association, the Crisfield Seafood Packers, the Point Pleasant Fisheries, the American Littoral Society, Office of Naval Research, American Museum of Natural History, Woods Hole Biological Laboratory of the Bureau of Commercial Fisheries, American Geographical Society, and the Guild of Creative Arts of Shrewsbury, New Jersey.

THE AMERICAN LITTORAL SOCIETY

During 1961 this laboratory has become a focal point for divers interested in conservation and underwater natural history. Dozens of divers, having lost enthusiasm for fish spearing and treasure hunting, have volunteered their services to us or requested guidance in natural history study. We have responded by helping them found the American Littoral Society, a national organization of amateur and professional naturalists. Membership, which is open to all active underwater observers who subscribe to the Society's objectives, is growing rapidly--it now numbers about 400. Incorporation is pending; local chapters are being established, and the first annual meeting is slated for March 1962.

The objectives of the Society are to encourage underwater study of shore life by direct observation of fishes and other marine animals; to collect, compile, and publish the records of members' observations; to assist members in solving problems of scientific study, identification and description; and to foster public information about shore life and public awareness of needs for conservation action.

The Society conducts periodic coastwide surveys similar to the bird counts of the Audubon Society. For these, standard recording forms are prepared and distributed and upon return are collated at Chapter or National Headquarters. Special instructions and forms are issued for specific missions. Results of the Society's first survey, a pilot study carried out in September 1961, gave encouraging results. Reports have come in from Maine to the Virgin Islands, with most from the Middle Atlantic coastal area. These will soon be published by the Society in a special survey volume. A full-scale program, covering both Atlantic and Pacific Coasts, will commence in 1962. Surveys are tentatively planned for May, July, and September.

John Clark was elected to be the Society's first president.

VOLUNTEER ASSISTANTS AND COOPERATORS

The cooperation and interest in our program by individuals, private organizations, State agencies and other institutions during the past year has been gratifying. We are fortunate in having eight volunteer cooperators actively assisting in research projects. Each assistant donates from 8 to 30 hours per week and adheres closely to a work schedule. Members of the staff have given lectures, demonstrations, and helped in conducting field trips for high schools and universities.

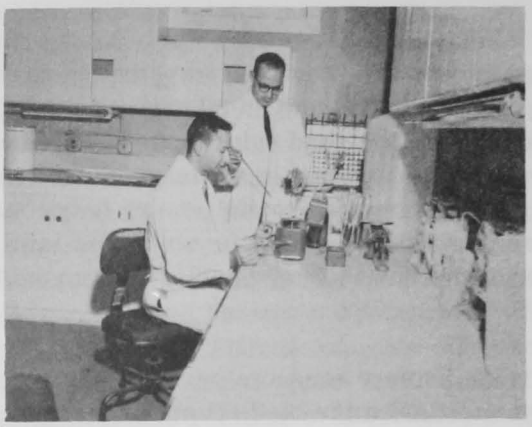
We have received many offers of volunteer assistance from students and institutions as well as from sportsmen who have offered us use of their boats.

Columbia and Rutgers Universities are using our facilities for graduate and summer programs.

These activities together with research opportunities for visiting scientists will expand this year.

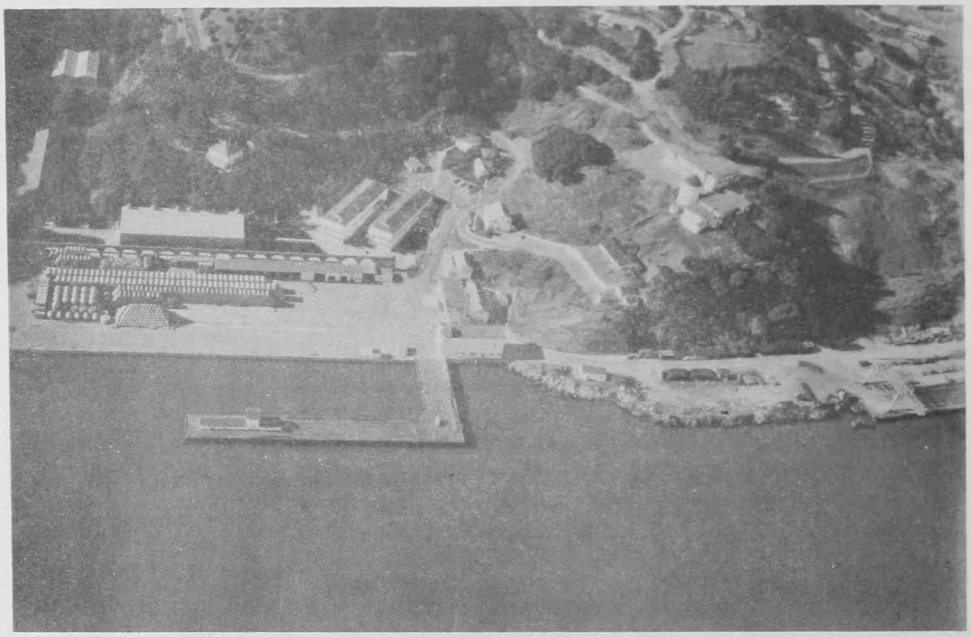
Sandy Hook laboratory and a local American Littoral Society team cooperated in this New Jersey Coast survey in December

Secretary Udall delivering the dedication address



Dr. Eisler (left) and Dr. Prager working in the medium preparation room of the microbiology laboratory

Tiburon Marine Laboratory
Tiburon, California



Tiburon laboratory

Through the cooperation of the University of California at Los Angeles space was provided for the research program during the first eight months of the year. Space requirements of the University necessitated that we vacate and in late August the U. S. Navy authorized a lease for occupancy of two buildings at the deactivated naval submarine net depot on the Tiburon Peninsula in north San Francisco Bay. Operations located at the University of California were then moved to the new site, to be known as the Tiburon Marine Laboratory.

The site of the new Tiburon marine laboratory in Marin County, north of San Francisco, is located in an area of extensive sport fishing for salmon, striped bass and species of bottomfish. It is located near the geographic center of marine game fishing effort on the Pacific Coast with extensive angling; to the south for

marine game species of yellowtail, albacore tuna, barracuda, kelp bass, marlin, and bonito; and to the north for species of chinook, silver and pink salmon, steelhead and many species of bottomfish.

Geographically, the Tiburon laboratory fills a gap in the Federal fishery research program for the little studied marine area from Monterey northward to the Columbia River. Extensive marine facilities are available at the Tiburon site, an excellent deep water dock and mooring bulkhead, with ample storage space and possibility of expansion to additional buildings. In view of projected needs, a request has been made to the U. S. Navy for use of additional facilities.

The laboratory is close to other research facilities in the greater San Francisco Bay area, such as the California Academy of Sciences, University of California, Stanford University and other colleges, universities and private facilities having common interests in marine research.

Initial operations were established at the Tiburon laboratory and a limited amount of rehabilitation of the assigned buildings has been completed.

Progress on development of a comprehensive marine game fish research program for the Pacific area was made during 1961. Current research programs now being conducted by the various State and Federal agencies have been examined to determine what type of marine research is being done, and where knowledge is lacking.

A number of meetings of direct concern to the marine research program were attended. State representatives were contacted, individually and collectively. Through the auspices of the Pacific Marine Fisheries Commission a special meeting was held at Portland, Oregon, in April, where the general objectives of the marine game fish research program were outlined to the fish and game agency representatives from the States of Alaska, Washington, Oregon and California.

During the year information was compiled on the extent, facilities, locations of fishing and species of marine game fish angling in the eastern and central Pacific. Information used in this survey was compiled through assistance of (private) sport fishermen and cooperation of State and Federal agencies. This information is being used in the drafting of 19 charts. These charts show the general areas and seasons of fishing, and species usually taken in the various geographical areas, and cover all locations of major marine game fishing effort from Mexico to Canada and in the States of Alaska and Hawaii.

Limited research was conducted with the cooperation of the U. S. Navy on the feasibility of recording sea surface temperatures from a moving helicopter.

Development of information on some little known but important species of sharks common to the areas of marine game fishing in Monterey Bay was also undertaken.

NORTH CENTRAL RESERVOIR INVESTIGATIONS
Yankton, South Dakota
Norman G. Benson, Chief

Yankton Staff
From left to right:--Eunice O. Lowe, Charles H. Walburg, Norman G. Benson, Ross V. Bulkley

The North Central Reservoir Investigations was established in November 1961 for studying the fish populations of the mainstem reservoirs of the Missouri River. These reservoirs are located in South Dakota, North Dakota and Montana, and will provide 1,191,000 surface acres of water by 1964. Water levels and water exchange rate in each reservoir are managed differently and the fish populations are extremely complex. The research program will be comprehensive although the principal objective will be to determine the fish population dynamics of these impoundments. A prospectus on the proposed sport fishery reservoir research program on a national level was published in 1961.

Our work will include: (1) development of methods of measuring population size and for sampling fish stocks; (2) measurement of variations in year-class strength, growth rates and mortality rates, and relating these variations to different types of water management and to ecological conditions; and (3) measurement of limnological characteristics and relating them to various types of water management and fish life histories. Both the tailwater and impounded areas will be studied. The program will involve major fish species in impoundments and not only those caught by sport fishermen. Research on fishery management techniques (e.g., fish control) will not be initiated until adequate knowledge is available on normal fish population characteristics.

One future phase of our program will be to compile available data on ecology and fish populations of large reservoirs throughout North America. The information will be placed on punch cards and analyzed for derivation of biological trends. This work will help in guiding future research and for making better use of available knowledge.

Office and storage space has been provided by the Army Corps of Engineers at the Gavins Point Dam Powerhouse near Yankton, South Dakota. The work during the early phases of this investigation will be principally in the field and extensive laboratory facilities will not be required.

Thus far we have been assembling available reservoir information, procuring equipment and planning the research program. Most field work during 1962 will be concerned with the determination of sound methods for measuring reservoir fish populations. The early limnological work will also be concerned with sampling and measurement techniques. Due to the dynamic nature of reservoir ecology, recording instruments will be used and modified for collecting continuous limnological data.

The rapidly changing conditions in reservoirs makes them more difficult to study or understand than natural lakes. For fishery management purposes, however, reservoirs hold greater promise than natural lakes because they are man-made and man-controlled. Extensive information on water volume, flow, temperature, etc., is routinely collected for other water management purposes. Many of the ecological variants can either be controlled or predicted and new techniques can be introduced, when required, for the benefit or detriment of various species.

ROCKY MOUNTAIN SPORT FISHERY INVESTIGATIONS
Logan, Utah
(Completion Report)

The Rocky Mountain Sport Fishery Investigations was terminated on October 31, 1961. A manuscript entitled "Equilibrium Yield and Management of Yellowstone Lake Cutthroat Trout" is being reviewed and will summarize population and life history information collected from 1949 to 1961.

In 1961, data were collected and analyzed on catch, spawning runs, fishing-for-fun, fry production and homing.

The 1961 catch was somewhat higher than the 1960 catch but was less than the record 1959 catch.

Spawning runs were about the same size as in 1960.

The fishing-for-fun program had little effect on the total catch and a significant mortality of fish hooked and released in mid-summer was indicated.

Predicted strength of the 1957 year class in Pelican Creek in 1961, based on lake water levels, was close to the actual value.

A high degree of homing was exhibited by spawners transplanted from stream traps back into the lake.

1961 CATCH FROM YELLOWSTONE LAKE

The catch of cutthroat trout in 1961 was estimated to be 312,594 fish. This was 5.6 percent greater than the 1960 catch and 20.6 percent less than the 1959 catch. The increase in catch over 1960 was accompanied by an increase in number of anglers and in fishing effort, but catch-per-man-hour was lower in 1961 than in 1960.

The 1961 catch of 312,594 fish is somewhat below the mean equilibrium yield of 325,000 fish which we have postulated for the lake. This yield of 325,000 fish can only be obtained on a sustained basis if the fish stock is in a healthy condition and has not been overexploited. As the population was seriously overexploited in 1959, the 1961 catch was excessive and further depleted the cutthroat stock. The fish population cannot be expected to recover as long as fishing pressure continues to increase unless measures for reducing the catch are put into effect.

SPAWNING RUNS

Age composition of the spawning runs was essentially the same as last year, although Pelican Creek had a higher percentage of old fish in the run than in 1960. The number of spawners in Chipmunk, Grouse, and Arnica Creeks was below the average for the past 12 years; Clear Creek was about average; and Pelican Creek and Cub Creek had above average runs. Fish stocks in the West Thumb are apparently being depleted more rapidly than the northern area stocks, as the three study streams with below average number of spawners support the West Thumb fishery.

FISHING-FOR-FUN PROGRAM

The National Park Service has instituted "Fishing-for-fun" program on Yellowstone Lake. In this program anglers are urged to release all trout they cannot use and to treat carefully all fish they plan to release. The program is voluntary and we have observed that some anglers regard the program as an excuse to continue fishing after catching their limit.

Participation

In 1961 we interviewed 2,661 anglers to determine the degree of participation in "fishing-for-fun". The interview data show that: (1) 80 percent of the successful fishermen kept all the fish they caught; (2) 65 percent of the anglers who returned fish still kept their limit; and (3) only 3.9 percent of the fishermen returned all fish captured.

There appears to be some "fishing-for-fun" philosophy present but it is used mainly as a method to continue fishing after the limit is

reached. Many anglers have always released trout caught in Yellowstone Lake, and it was not possible to determine how much influence the 1961 "fishing-for-fun" publicity has had on the fishing public. The program and interviews should be continued.

Hooking mortality studies

The encouraging of "fishing-for-fun" may induce a hooking mortality on the fish stock that was present to a lesser degree in the past. Hooking studies were conducted on June 17-18 (during the spawning season) and on July 22-23 (after the spawning season). Barbless and barbed treble and single hooks were used on medium-size spoons. These are the most common type of artificial lures used on Yellowstone Lake. The fish were held for five days after hooking.

In the June experiment 556 fish were caught and only 4 fish (0.7 percent) were killed. These were killed by both barbed and barbless treble hooks but none by single hooks.

In the July experiment 234 fish were caught and the average mortality for all lures was 21.3 percent. Only treble hooks were used but the barbed hooks caused a 4.4 percent higher mortality than the barbless hooks. The increase in mortality in July over June was due to the fact that the fish were more active and were harder to catch and release. In addition, the water was warmer and fungus more prevalent. In fact 24 percent of the survivors showed some fungus, indicating that the total hooking mortality was probably higher than 21.3 percent. The fishing in June was entirely from the shoreline while fishing in July was from canoes. This factor introduced a possible error in the estimates, but this transition from shoreline to boat angling is typical of the seasonal change in type of fishing in Yellowstone Lake.

These two experiments are not conclusive and should be repeated, but they do indicate that there is a substantial mortality introduced when fish are caught and released after the spawning season. They suggest that there should be special lure restrictions for anglers who fish-for-fun.

ESTIMATES OF PELICAN CREEK YEAR-CLASS STRENGTH

Estimates of Pelican Creek year-class strength have been made from fluctuations in Yellowstone Lake water levels during the spawning and incubation period. The number of initial spawners from a year class is used as a measure of year-class strength. Year-class strength in the Fishing Bridge area fishery is similar to Pelican Creek, and the relative abundance of fish available to this fishery is indicated by the forecasts.

Data are now available to check the estimate of the 1957 year-class. Predictions for the 1957 year-class which entered the 1960 Pelican Creek spawning run at age III and the 1961 run at age IV indicated a below average year-class. The best estimate of the number of fish that would return as initial spawners from the 1957 year-class was 7,121 fish, with 95 percent confidence limits of 4,970 to 10,200 fish. Analysis of data collected in 1960 and 1961 showed that 8,709 fish from this year-class spawned during these years. The actual number was 22 percent above the predicted number but was well within established confidence limits. This agreement between forecast and actual returns supports the hypothesis that production of cutthroat trout in Pelican Creek and adjacent spawning areas is largely controlled by water levels during the spawning and incubation season. Even a large spawning run such as 1957 cannot produce a strong year-class unless water levels are low and relatively stable during spawning and incubation.

HOMING EXPERIMENTS

Use of the number of spawners in key streams as a measure of the condition of the fish stock depends upon a strong homing instinct or knowledge of factors which cause straying. Data collected from some spawning runs suggest straying is more common than originally suspected.

A homing experiment, first carried out in 1955, was repeated in 1961 at Clear and Cub Creeks. Spawners entering traps in the two streams (1 mile apart at mouth) were trans-

ported to three different areas of the lake: (1) midway between the two streams; (2) near mouth of the adjacent stream; and (3) near the center of the lake (from 3 to 7 miles from both streams). Approximately 150 fish were released in each area, or 450 fish from each stream.

A strong homing instinct was exhibited by marked fish which were recovered, as an average of 82 percent of the recovered fish in the two experiments returned to their parent stream. Only 11 percent in 1955 and 22 percent in 1961 of the returns were recovered in the adjacent stream. Returning fish were passed upstream above the traps and it is possible that some of the fish recorded as strays would have moved back downstream and returned to their parent stream if allowed. Area of release did not influence the degree of straying or the percentage recovery of marked fish.

ACCOMPLISHMENTS OF ROCKY MOUNTAIN SPORT FISHERY INVESTIGATIONS

1. The general life history of cutthroat trout in Yellowstone Lake was described. These studies included growth rates, mortalities, movement and migration patterns, food and feeding, predation, homing, racial work, spawning habits, spawning runs, and an evaluation of factors influencing reproduction.

2. A complete creel census was conducted from 1950 to 1961.

3. Yellowstone Lake was mapped and the general ecology was described.

4. Population dynamics of the cutthroat trout population was analyzed in respect to rates of exploitation, variations in year-class strength, equilibrium yield, and management.

5. A method was developed for predicting year-class strength during the first year of its existence for the Fishing Bridge area.

6. Methods were developed for measuring and evaluating the condition of the fish stock from either the catch or spawning runs.

7. Studies were made on the effects of airplane spraying of DDT for spruce budworm control on the fish and fish foods in trout streams in Yellowstone Park and in western Montana.

8. The life history of Grebe Lake grayling was described.

9. A study of stocking and management of the Madison River System was completed.

10. Results of the work are reported in 31 publications.

FUTURE NEEDS ON YELLOWSTONE LAKE

The large natural fluctuations in cutthroat trout production in Yellowstone Lake together with the present over-exploited condition of the stock make continuous observation of the population imperative in order to maintain adequate spawning runs. Study should continue on age composition and size of the catch and spawning runs, fishing-for-fun program, influence of exotic fish on the cutthroat stock, and factors limiting cutthroat production.

ACKNOWLEDGMENTS

Much of the success achieved by the Rocky Mountain Sport Fishery Investigations in Yellowstone Park was made possible by the excellent cooperation and support received from personnel of the National Park Service. We wish to express our appreciation to them for their continued interest and support. We are also indebted to the Branch of Fish Hatcheries for assistance of their personnel in collecting data and for use of buildings and equipment during the study.

Dr. Oliver B. Cope supervised this investigation from its inception to its closing. Other permanent biologists concerned with the project were Mr. Harvey Moore, Mr. Orville Ball, Dr. Martin Laakso, Mr. Ross V. Bulkley and Dr. Norman G. Benson.

ENVIRONMENTAL INFLUENCES

FISH CONTROL LABORATORY
La Crosse, Wisconsin
Robert E. Lennon, Chief

La Crosse Staff
Front row-left to right:--D. A. Redmond, R. E. Sampson, D. C. Payne, R. E. Lennon
Second row-" " " C. R. Walker, B. L. Berger, P. S. Parker, T.E Kennedy
Third row- " " " R.E. Shawley, D. F. Mair

HIGHLIGHTS OF 1961

Major construction and rehabilitation jobs on buildings were completed in December. Some minor jobs on services, fittings, and equipping remain to be done.

Problems with water quality continue. A shallow well was drilled and tested.

The general chemistry and biochemistry laboratories were staffed and equipped. Preliminary experiments on fish and facilities were made.

A fish-herbicide project was initiated and experimental facilities were constructed.

A southeastern Fish Control Laboratory was planned for location on the property of the Warm Springs (Ga.) National Fish Hatchery.

CONSTRUCTION AND REHABILITATION

A large amount of construction and rehabilitation was completed. Major items of work included:

1. A fish holding house, 82'x46', masonry, including a small public aquarium was built. It was placed in use in the second quarter.

2. A 24-inch culvert, 80 feet long, and a stoplog structure were constructed between the bass pond and the La Crosse River.

3. The La Crosse River levee was cleared, widened, and graded. A gravel surface roadway was constructed. Extensive riprap was placed on the river side of the levee to arrest serious erosion and undermining.

4. A 10-inch test well, 50-feet deep, was drilled and tested at 300 gpm for 60 days in an attempt to find a better supply of water. In addition, 7 test points were driven elsewhere on the grounds at depths of 5 to 25 feet, and the waters were tested.

5. Nine 1/100-acre, concrete pools were constructed on the levee for fish-herbicide experiments.

6. A cyclone fence was erected to surround the daphnia pools and fish-herbicide pools.

7. Mortar on the exterior of the laboratory building and shop-garage was pointed.

8. Laboratory property in Riverside Park was surveyed to establish fixed and marked boundaries. The original deeds on two parcels are being rewritten in cooperation with the City to remove restrictive clauses.

9. The shop-garage was rewired to meet electrical codes and to provide additional service.

10. The second floor of the laboratory building was remodeled and a west wing constructed. Offices, a library, new biochemistry, special-use and physiology laboratories were placed in use during the fourth quarter.

11. The diagnostic laboratory operated by fish hatchery biologists was renovated and its facilities expanded.

12. The old fountain-fish pool was removed and the site was graded.

13. New concrete curbs were installed to the east and south of the laboratory building and the lawns were repaired.

14. A steel guard rail bordering the Park Drive in front of the laboratory building was erected.

15. A masonry oil-chemical storage building was built.

16. Driveways and parking areas were laid out, graded, and surfaced with crushed rock in preparation for future paving.

17. A new telephone system and expanded automatic fire detector-alarm and intercom systems were completed and put into use in the fourth quarter.

OPERATIONS

Water supplies

Continuing difficulties were experienced with city water and well water during the year. Much effort was expended to determine the specific undesirable components, the methods to eliminate or counteract them, and their effects of equipment and on test fishes. Consultants included degassing, aerating, mechanical filtering, charcoal filtering, and softening among the processes necessary to make the water suitable for use. Obviously, the costs and space requirements for processing large volumes of water are beyond us. Experimental treatments of fractions of the city water and well water are continuing in attempts to meet the more critical needs.

In addition to its use for domestic purposes at the laboratory, the city water is preferred over well water for certain operational needs because of its high pressure (100 psi). It has, however, high concentrations of carbon dioxide, chlorine, ferrous and ferric iron, and sludge. Despite softening and carbon filtering, it gives poor results in our large deionizer unit. A more elaborate pre-treatment is necessary before the deionizer can produce the volumes of water necessary for bioassay work. Furthermore, experimental lots of eggs from rainbow trout failed to hatch in city water which had been carbon filtered and aerated.

The water from our 140-foot well contains ferrous and ferric iron, high sulfates and chlorides, manganese, and ammonia nitrogen (range: 9-29 ppm). Iron bacteria are abundant and a host of protozoans were present during tests in the spring and fall. The ammonia nitrogen reduces the efficiency of a large aerator in removing carbon dioxide and adding oxygen, and it reduces the fish-holding capacity of tanks and troughs.

Iron bacteria sludges have clogged water lines and taps, thereby destroying experiments in progress. Brook, brown, rainbow and lake trout hold reasonably well in the water provided tank loadings are light. A 60-percent hatch of rainbow trout eggs was obtained during a test of water quality. Warmwater fishes, on the other hand, hold poorly in the deep well water which qualifies their usefulness for bioassays. The accepted solution of the problems connected with this water was to seek a new source.

A 10-inch test well, 50-feet deep, was drilled near our west boundary in June, relatively close to the confluence of the Mississippi, Black, and La Crosse Rivers. The hope is to induce a flow of ground-cooled and filtered river water in to the well. Test pumping at 300 gpm for 60 days, however, did not draw in river water. Iron and iron bacteria are absent, ammonia nitrogen ranged between 1 and 8 ppm, and manganese fluctuated between 3.4 and 5.3 ppm. Two species of trout and 24 species of warmwater fish held well in this water for a 2-month period, but tank loadings were light.

Seven, 1.5-inch test points were driven to depths of 5 to 25 feet on the laboratory grounds in attempts to locate water free of iron and manganese. None was found. Some of the greatest concentrations of iron occur at shallow depths near the river bank.

Analyses were made on river water concurrently with those on the well waters. The chemical quality of the river water is good. Ammonia nitrogen occurs at less than 1 ppm, iron as a trace, and manganese as a trace. Its usefulness, however, is limited because the turbidity is high, and the temperature during the summer ranged up to 84°F.

As a result of the prospecting and extensive testing, a decision was made to develop the 10-inch, shallow well, to produce 700 gpm. The water is not wholly satisfactory but it is significantly better than that of the deep well. Furthermore, the higher rate of pumping might cause an inflow of river water. A contract for the development was advertised in December and the work will begin early in 1962.

Several 50-gpm diatomite filters were obtained as surplus from the Army and Air Force in December. They are being tested with the intention of employing them in the deionized water system, in the aquarium supply system, and in a portion of the fish-holding system.

Screening of chemicals

Thirty species of fish were acquired from hatcheries or natural waters and were held at the laboratory to determine their suitability as test specimens. Some were used in tests of water quality; many were used in the preliminary tests which are necessary to the bioassay program. Specimens were held in bioassay vessels in reconstituted deionized water and in well water, at warm and cold temperatures, and at various concentrations of dissolved oxygen. Considerable information has been obtained on the chemical and thermal acclimatization of fish to bioassay conditions. For example, the oxygen requirements of small rainbow and brown trout, goldfish, yellow perch, and green sunfish have been studied relative to establishment of loading capacities in test vessels. Condition factors (K) have been computed for young fish of several species to provide limits on the sizes of fish which can be used in the screening program.

The hematocrit levels of test fish might be used as indicators of physical condition. Observations were made on the utility of commercial heparinized capillary tubes for microhematicrit determinations in 14 species of warmwater fish. It was demonstrated that the anticoagulant in the capillaries was not sufficient to prevent clot-

ting of blood samples from 13 species. A manuscript on this work was prepared.

Following completion of major construction activities in December, a trial run of the basic screening procedure was made. A rotenone product was used as the test chemical. The procedure proved to be generally satisfactory with only slight modifications necessary.

Installation of fiberglass screen holders in the fiberglass experimental troughs was one of the modifications needed to facilitate the bioassay program. The epoxy resin which was used as a cement turned out to be highly toxic to rainbow trout and goldfish. An investigation disclosed the resin became non-toxic after extended curing and flushing with water. A brief report on this problem was published.

Intensive testing of chemicals

Basic facilities of the biochemistry laboratory were finished in December. Additions to the electrical service are needed and a contract for this work has been awarded.

A biochemist and a physical science aid entered on duty during the fourth quarter and they have been organizing and equipping the laboratory for early use. Some of the scientific equipment has been acquired from GSA surplus stores.

Physiological investigations

The physiology laboratory was included in the second-floor wing which was added to the main building. The basic facility is complete except for some upgrading of electrical services. Staffing and equipping will be done in fiscal year 1963.

Tests of electrofishing gear in the field

The 12- and 18-foot electrofishing boats were employed in 23 trials. Operations with AC and DC power units were conducted night and day in hard and soft waters, turbid and clear waters, cold and warm waters, and at high and low water levels. The trials demonstrated that there is much to be done to make electrofishing gear more generally and more efficiently applicable to large waters. The performances showed sufficient promise under difficult conditions to warrant an increase of experimentation. Added emphasis will be given therefore to the electrofishing studies in 1962, since we must rely upon this means to collect certain fishes in natural waters for use in bioassays.

Assistance was given on request to the Minnesota and Wisconsin conservation departments by providing specimens for exhibits at State Fairs, to the Genoa National Fish Hatchery in collecting brood-size bass from the Mississippi River, and to the Branch of Fishery Management Services in surveying trout populations in ponds and streams on the Camp McCoy Military Reservation.

Fish-herbicide investigations

This project has been initiated in cooperation with the Fish-Pesticide Research Laboratory. Its object is studies on the long-term effects of herbicides on fish. We intended at first to utilize two earthen ponds at the Genoa National Fish Hatchery, but an investigation of them showed that extensive and expensive alterations were needed.

Nine 1/100-acre concrete pools were built on the La Crosse River levee as experimental facilities. Water supply lines were laid and access roadways to the front and rear sides of the pools were made and surfaced with crushed rock. A cyclone fence was erected around the pool area.

The pools are designed for seasonal use, i.e., early spring through late fall. Loam is placed on the bottoms of the pools to support growths of selected aquatic plants. Herbicides will be tested at several dosages and frequencies of application for their effects on the fish, the plants, and the soils. In particular, the influences of herbicides and residues on survival, growth, and reproduction of fish over long periods of time will be noted.

The first experiments will begin in the spring of 1962.

OTHER

The Southeastern Fish Control Laboratory

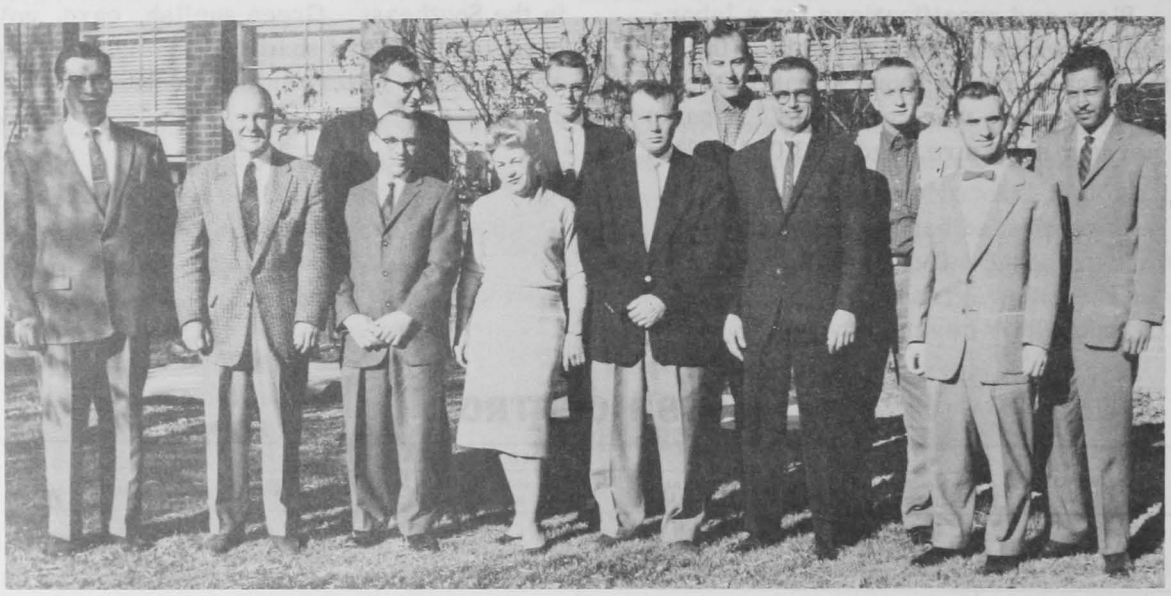
A site for the Southeastern Fish Control Laboratory was selected at the Warm Springs (Georgia) National Fish Hatchery in August. Plans and specifications for a laboratory building which closely resembles the one at the Fish Farming Experimental Station, Stuttgart, Arkansas, were prepared. A pump house, aerator structure, and a 13,000-gallon, redwood reservoir are included. A contract for construction will be awarded early in 1962. Fish-holding, garage, and shop facilities will be erected in fiscal year 1963.

The laboratory will be concerned in general with problems of fish control in warm waters, and in particular with control problems in the Southeast. Green sunfish, carp, goldfish, gars, and gizzard shad are considered undesirable in many waters in that section of the country.

FISH CONTROL

PURPOSE	METHODS				
	RADIANT ENERGY - LIGHT, HEAT, ELECTRICITY, SOUND	PHYSICAL AND MECHANICAL	CHEMICAL AND BIOCHEMICAL	GENETICS	BIOLOGICAL AGENTS
INDUCE SPAWNING	Electric shock Controlled light Sound stimulant	Hydraulic control	Hormones	Select for season and duration of spawning	Plant introductions
PREVENT SPAWNING	Electric shock Radiation Controlled light Sound depressant		Sterilants Hormones	Select for low fertility	Plant introduction or removal
IMPROVE GROWTH, VIGOR, FECUNDITY, DISEASE RESISTANCE	Light control			Brood stock selection Select for wildness Select for rapid growth Select for disease resistance	
TRANSPORT	Electro-narcosis	Aeration Hydraulic control Temperature control	Anesthetics Sedatives Decontaminants Diet manipulation		
PREVENT OSMOTIC SHOCK		Acclimatization	Osmoregulatory compounds	Select for adaptability	
PREVENT ENTRY	Electrical barrier	Barrier Hydraulic manipulation	Repellants		
RESTRICT MOVEMENT	Electrical array	Hydraulic manipulation	Attractants Repellants	Select for non-migratory strain	
SELECTIVE REDUCTION	Pulsed current	Water level manipulation Gear development	Selective toxicants		Selective infectious disease Selective parasites Predator introduction Competitor introduction
FACILITATE CAPTURE	Sonic attractant Electrical guiding array	Water level manipulation Gear development	Attractants	Select for catchability	
ERADICATION	Lethal current	Gear development	Lethal compounds		

FISH-PESTICIDE RESEARCH LABORATORY
Denver, Colorado; Jackson, Wyoming; Tishomingo, Oklahoma; Patuxent, Maryland; Marion, Alabama
Oliver B. Cope, Chief



Staff
Front row-L.to R.:--J. O'Donnell, O.B. Cope, H. Sanders, Mrs. D. Harding, W.R. Bridges, Paul Frey, C. Van Valin.
Back row- L.to R.:--B. Kallman, A. Andrews, G. Wallen, D. Allison, E. Davis.

DDT-cutthroat chronic effect study at Jackson, Wyoming, was continued through the year.

2, 4-D-bluegill chronic effect study at Tishomingo, Oklahoma, was started in July and was still in progress at the end of 1961.

A DDT study in Blackburn Pond near Denver was started in July and was still in progress at the end of the year.

Field studies were conducted on tent caterpillar in Alabama; alligatorweed in South Carolina; elm spanworm in North Carolina; spruce budworm in Montana; alfalfa weevil in Wyoming; and fire ant in Florida.

Bioassay studies were made at Denver to measure effects of temperature and exposure time on toxicity of kepone and heptachlor to red-ear sunfish.

Much work was done on methodology and many determinations made with assay for insecticides with houseflies and Drosophila and for herbicides with cucumber seeds at Denver.

Progress was made on improvements in chemical analytical techniques and hundreds of residue analyses were done in the Denver chemistry laboratory.

Many residue analyses and fish toxicity determinations were performed for other agencies.

Construction of ponds was completed at Marion, Alabama, and Tishomingo, Oklahoma.

Construction of an annex to the chemistry laboratory in Denver was at an advanced stage at year's end. A new aquarium room at Denver was completed.

Construction of jar-hatching facilities was near completion at Jackson, Wyoming.

FACILITIES

Five experimental ponds, 1/10-acre in area, were built at the Bureau station at Marion, Alabama. Seeding of banks has been accomplished, and preparations have been made for subdividing the ponds for replications in experiments. These ponds, together with new portable plastic ponds, provide the staff with excellent facilities for exposing fish to pesticides in a variety of ways.

Six experimental ponds, 1/10-acre in size, were built at the National Fish Hatchery at Tishomingo, Oklahoma. Banks have been stabilized and final arrangements are being made to begin the next long-term experiment. These ponds supplement the three older ponds and the three raceways now being used.

The 20 x 27-foot addition to the chemistry laboratory at Denver is scheduled for completion in January 1962. This new space will relieve crowding in existing laboratories and provide for additional equipment and staff.

The 550-square foot aquarium room completed this year at Denver has enabled us to quadruple our ability to perform fish bioassay tests under well controlled conditions.

Fabrication of units to hatch and culture trout in jars at Jackson, Wyoming is nearing completion. These units will be used to follow the fates of progeny of adults exposed to DDT since December 1960.

EXPERIMENTAL FIELD STUDIES

DDT in Swan Creek

In 1960 studies were made on the effects of a spruce budworm DDT airplane spray in the Gallatin River drainage in Montana. Measments on a tributary, Swan Creek, showed that a drastic kill of aquatic invertebrates took place, leaving the sprayed portion of the stream essentially devoid of bottom organisms.

In 1961, to learn about the recovery of invertebrates in the same stream sections, we hand-sprayed DDT on the stream at approximately the same rate applied by airplane in 1960. Samples of dead and dying invertebrates drifting in the stream were then collected, as in 1960. Although the 1960 and 1961 treatments were not strictly comparable, it is clear from drift samples that considerable increase in aquatic insect populations took place between the two sprayings.

Table 1 shows the numbers of insects taken in drift samples at various times after the spray, in each of the two years. A noteworthy change in population composition was the failure of Baetis to recover as completely as did some other forms, and the development of relatively large numbers of the stonefly Hastaperla.

Analyses of water and insect samples showed that the effect of the DDT was felt for only a short distance downstream from the sprayed area.

DDT in Blackburn Pond

Blackburn Pond near Denver was treated with DDT at the relatively low rate of 0.02 ppm in July for a study of the breakdown of the compound in a warmwater pond. Sampling of water, rainbow trout, bullheads, crayfish, aquatic vegetation, and bottom sediments was carried on after the application, and the samples were measured for residues of DDT and its metabolites. The following conclusions were reached in these studies:

Table 1:--Comparison of the number of aquatic insects per drift sample after DDT applications in 1960 and 1961, Swan Creek, Montana

Order of Insects	Time elapsed after DDT application, in hours															
	0.1		0.25		0.5		1.0		1.5		3.0		6.0		24.0	
	1960	1961	1960	1961	1960	1961	1960	1961	1960	1961	1960	1961	1960	1961	1960	1961
Plecoptera	257	-	-	1375	1700	3160	450	3947	450	2571	161	204	22	84	3	14
Ephemeroptera	331	-	-	18	2134	115	17500	269	10735	387	4582	111	325	163	110	35
Trichoptera	87	-	-	100	259	546	1295	865	1840	610	479	92	80	85	18	6
Coleoptera	1	-	-	0	0	1	0	0	80	1	5	0	1	0	0	0
Diptera	59	-	-	1708	142	367	225	452	525	280	799	11	118	12	19	5
Total Numbers	735	-	-	3201	4235	4189	19470	5533	13130	3849	6029	418	549	344	150	60

The concentration of DDT in the pond water was at its highest level 30 minutes after treatment. A decline in DDT levels then took place, until none could be detected 21 days later.

Aquatic vegetation contained 6 to 30 ppm of DDT + DDE + DDD during the first week after treatment and declined to 1 ppm in 65 days.

In the bottom mud there was 8.3 ppm of the DDT complex after 24 hours. After the third day the concentrations were 1.5 ppm and below.

Bullheads and trout contained the greatest amounts of chlorinated hydrocarbon 30 to 40 days after treatment, with concentrations over 4 ppm. A decline in levels took place after that time, but trout contained about 2 ppm after 120 days. Table 2 shows the residues found in trout and crayfish, and indicates the development of higher residues in the trout than in the crayfish. Bullheads contained about the same residues as the trout.

Few acute effects followed the treatments. A few dead trout were found within the first four days and only small numbers of aquatic insects were affected. No dead crayfish were seen. DDD levels in the fish were

Table 2.--DDT, DDE, and DDD residues, in ppm, in rainbow trout and crayfish in Blackburn Pond after addition of 0.02 ppm of DDT to the water.

Days after Treatment	Unaffected Rainbow Trout				Unaffected Crayfish			
	DDT	DDE	DDD	Total	DDT	DDE	DDD	Total
2	0.62	0.43	0.14	1.19	-	-	-	-
3	0.96	0.22	0.13	1.31	-	-	-	-
6	1.56	0.70	0.19	2.45	-	-	-	-
14	2.50	0.62	0.26	3.38	-	-	-	-
21	1.27	1.01	0.52	2.80	0.81	0.55	0.45	1.81
26	1.90	1.15	0.57	3.62	0.36	0.72	0.39	1.47
33	1.90	1.30	0.77	3.97	-	-	-	-
35	2.70	1.24	0.74	4.68	-	-	-	-
42	-	-	-	-	0.56	0.56	0.35	1.47
49	1.67	2.33	0.74	4.74	-	-	-	-
56	0.93	1.31	0.68	2.92	-	-	-	-
65	0.77	0.89	0.65	2.31	0.29	0.53	0.68	1.50
85	0.79	1.48	0.84	3.11	-	-	-	-
91	0.27	0.73	1.10	2.10	-	-	-	-
98	-	-	-	-	0.11	0.15	0.07	0.33
105	-	-	-	-	trace	0.36	trace	0.36
112	-	-	-	-	trace	0.27	trace	0.27
120	0.88	0.71	0.96	1.95	trace	0.36	trace	0.36

relatively higher than in fish from other studies; after a month, the DDD in the Blackburn Pond fish was 15-52 percent of the total chlorinated hydrocarbon content.

DDT and cutthroat trout at Jackson, Wyoming

Studies on chronic effects of DDT on cutthroat trout began in December 1960 and continued through 1961. In this work, five lots of fish were given DDT once a week in their pelleted diets at a different rate for each lot, five lots were given DDT once a month in bath form, and one lot was furnished no DDT. Samples were withdrawn at intervals for chemical analysis for DDT and its metabolites, for hematology measurements, for size measurements, and for histological examination. Records were kept on day-to-day mortalities in the various lots.

Results of whole body residue analyses are presented in figure 1 and table 3. The figure shows residues of DDT, DDD, and DDE in fish fed DDT weekly in the diet, and demonstrates the relatively large amounts stored in the fish fed 3 and 1 mg. per kilogram of body weight. Fish fed the three lowest levels contained the insecticide in amounts not much different than those in the control group. The table shows residue measurements in fish given DDT monthly in baths. It is seen that fish bathed in the two highest levels stored the most insecticide and that the lower three levels resulted in stored amounts similar to those in the control fish.

In general, the rate of metabolism of DDT to DDE seems to be inversely related to the concentration of DDT to which the fish were exposed. A possible second metabolite DDD, has been found in almost all fish examined but it is as yet uncertain whether this substance is actually derived from the administered DDT. As reported in quarterly reports during 1961, DDT, DDE and DDD are all present in the basic ration fed at Jackson, derived primarily from cod liver oil which is in the feed. In some of the experimental fish receiving high levels of DDT, DDD has accumulated in amounts higher than found in controls or other experimental fish. It would appear probable that this accumulation is the result of metabolic action on DDT. Preliminary work indicates that various organs accumulate and metabolize DDT at different rates. Thus, brain appears to accumulate higher concentrations of total chlorinated hydrocarbon than does whole body. In brain also, more of the total is present as DDE than is the case with whole body.

Table 3.--Total chlorinated hydrocarbons in whole trout in parts per million. Fish were treated with(05 hr.)aqueous bath at indicated DDT concentration, at monthly intervals.

Lot	DDT Treatment	Days After First Bath											
		3			55			111			220		
		ppm	± 1/	N 2/	ppm	±	N	ppm	±	N	ppm	±	N
I	None	0.4	-	-	0.7		-				0.75		-
II	1.0 ppm	1.23	0.60	3	2.03	0.35	3	1.91	0.43	3	4.17	1.39	3
III	0.3 ppm	1.61	0.60	3	1.85	0.99	3	2.58	0.86	5	3.35	2.96	3
IV	0.1 ppm	0.76	0.25	3	1.37	0.49	3	1.75	0.61	5	2.28	0.19	3
V	0.03 ppm	0.71	0.03	3	0.78	0.17	3	0.86	0.17	3	1.47	0.13	4
VI	0.01 ppm	0.58	0.09	3	0.97	0.55	3	0.94	0.30	4	1.17	0.29	4

1/ Confidence limits, 0.05

2/ Number of fish analyzed

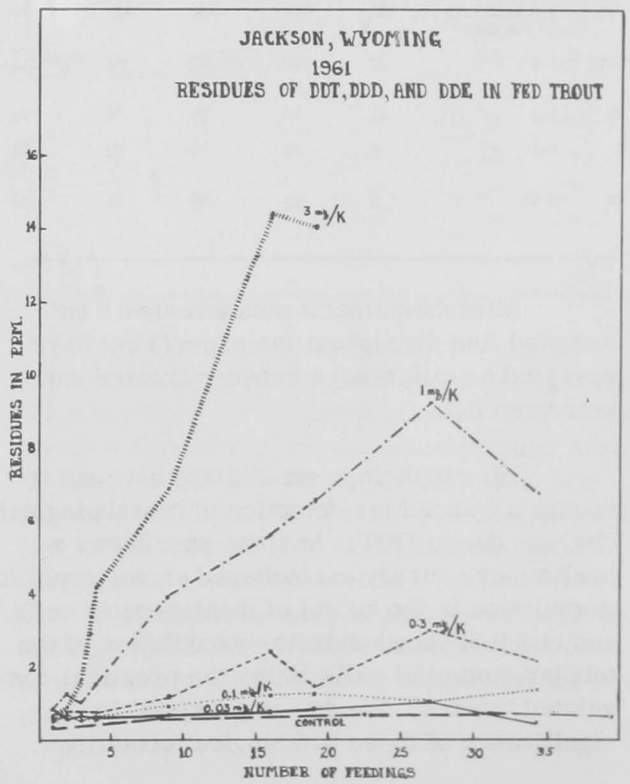


Figure 1:--Residues of DDT, DDD, and DDE measured in cutthroat trout from Jackson, Wyo. Trout were fed DDT in pelleted feed at 5 rates at weekly intervals.

Mortalities occurring in the different lots because of stresses of various kinds are summarized in table 4. The data show that through the first 11 months of the study, the highest mortalities took place in the two highest treatment lots of both the fed and the contact fish. The greatest numbers of deaths occurred in the lot fed 3 mg. per kilogram of body weight.

Table 4:--Cumulative mortality of cutthroat trout exposed to DDT at Jackson, Wyoming.

Lot	Treatment	Months After First DDT Treatment 3	5	7	9	11
		Numbers of dead trout				
I	None	11	35	50	57	90
II	1.0 ppm bath	31	190	286	292	304
III	0.3 "	16	182	280	288	301
IV	0.1 "	20	91	154	158	186
V	0.03 "	16	49	69	87	113
VI	0.01 "	13	49	68	90	138
VII	3.0 mg/K body wt. in diet	291	419	431	432	447
VIII	1.0 "	37	262	303	305	311
IX	0.3 "	11	46	74	86	99
X	0.1 "	6	28	49	63	88
XI	0.03 "	9	39	58	64	90

Microhematocrit measurements on sampled fish throughout the experiment have revealed no differences between treated and untreated fish.

Histopathology studies are not yet far enough advanced for detection of morphological changes due to DDT. In some specimens a preliminary survey encountered an eosinophilic granulation in the nuclei of many hepatic cells, and one fish shows extreme vacuolation of the tubular epithelial cells lining the proximal convoluted tubules. We do not yet know the significance of these pathological conditions.

Important differences in growth have developed among the various lots of trout. Table 5 presents average weights of trout in the 11 lots, measured at intervals during the year of treatment. The fish in lots receiving greatest amounts of DDT had the greatest average weights. Since the highest mortalities also occurred in lots receiving the most DDT, it is suggested that either alleviation of crowding or selection of less vigorous individuals accounts for the differences in growth. We feel that crowding was never an important factor in this experiment and that probably DDT was responsible for mortality in the smaller or weaker fish, leaving the larger or stronger ones to survive and grow at faster rates than the untreated fish.

Table 5:--Average weights, in grams, of fish in 11 lots of cutthroat trout treated with DDT at Jackson, Wyoming, beginning in December 1960

Lot	DDT Treatment	Sampling Dates 12/2/60	2/10/61	5/6/61	7/28/61	10/20/61	12/16/61
I	None	64.3	77.3	101.3	120.7	155.7	174.6
II	1.0 ppm bath	63.3	81.0	110.7	152.7	202.0	226.7
III	0.3 " "	63.3	78.3	109.3	142.7	194.3	216.7
IV	0.1 " "	63.7	76.0	102.3	128.0	166.3	193.0
V	0.3 " "	64.7	79.3	107.0	127.0	167.0	188.0
VI	0.01 " "	63.7	80.0	105.7	124.7	166.0	189.3
VII	3.0 mg/k body wt. in diet	63.3	78.7	113.7	162.0	213.7	240.0
VIII	1.0 "	64.3	77.7	100.3	137.7	185.7	202.7
IX	0.3 "	65.0	77.7	102.0	120.0	155.7	172.3
X	0.1 "	63.3	76.7	100.7	121.7	156.7	172.7
XI	0.03 "	63.7	77.7	100.7	121.7	158.3	181.3

2, 4-D and bluegills at Tishomingo, Oklahoma

An experiment to measure chronic effects of 2, 4-D on bluegill sunfish in treated ponds was begun at Tishomingo, Oklahoma, in July. Three ponds were partitioned with polyvinyl chloride sheeting to provide 6 testing spaces for fish. Each subdivision measures 1/10 acre. One space was used for the untreated control and each of the others was treated with one of 5 concentrations of Esteron 99, propylene glycol butyl ether ester of 2, 4-D. The treatment levels were: 10, 5, 1, 0.5, and 0.1 ppm. Mortality among fish was very small in the ponds with highest levels and none occurred in the lower levels. Mortality was seen only in the first 7 days of exposure. There appears to be a tendency toward faster growth in the lots of fish subjected to the most 2, 4-D. Spawning was

delayed for two weeks in the 10 ppm treatment; all other lots spawned at the normal time. Fry production appeared to be essentially the same in all lots. Microhematocrit readings do not differ among lots of fish.

Examination of sampled fish for histopathological changes is proceeding at a commercial laboratory; no results are reported yet. Analysis of 2, 4-D residues in sampled fish is being attempted in another commercial laboratory and no results are yet available.

Control of the aquatic weeds in the treated ponds varied in effectiveness. The pond treated with 10 ppm had 80-100 percent control of Chara, Potamegeton, Najas, Digitaria, Salix, and Typha. Some regrowth of Chara took place after 12 weeks. Death of weeds in other ponds varied from 0 to 100 percent, depending upon the kind of weed and the treatment level.

Esteron remained in pond water near treatment levels for about 3 weeks and all disappeared from the water after 12 weeks.

LABORATORY STUDIES

Chemical assay

Processing of water samples:--The processing of water samples containing insecticides has been a major problem in this laboratory. Samples collected in glass or plastic containers do not give satisfactory results because of losses before extraction, and charcoal-filled columns as described in the literature have yielded variable recovery rates.

As modified here, granular activated carbon is used to remove the insecticide from the water. The carbon is then extracted with a mixture of ethyl ether and petroleum ether; the extract is evaporated, purified by passage through an MgO-celite mixture, and chromatographed on paper.

Two methods of carbon treatment have been used in our studies, one in which the water sample is run through a bed of carbon, and the other in which the carbon is placed in the water sample and is mixed thoroughly by shaking or stirring.

No clear advantage of one method over the other can at present be seen regarding recovery of heptachlor epoxide. On the basis of a limited number of experiments, however, it is apparent that heptachlor is recovered in higher yield by mixing and shaking the carbon in the water. Heptachlor and heptachlor-epoxide recovery with the carbon column method is adversely influenced as the amount of insecticide is increased, but this is apparently not true with the shaking method. Table 6 has selected data which will serve to illustrate.

Table 6.--Recoveries of heptachlor and heptachlor-epoxide from treated waters, comparing the shaking method with the column method.

Method	Volume and rate	Insecticide Added		Percent Recoveries	
		Heptachlor ug.	Heptachlor-Epoxide ug.	Heptachlor	Heptachlor-Epoxide
Shaking with 10 g. carbon	1 gallon	25	25	55	92
		50	25	50	70
		100	25	58	71
		500	25	69	90
	5 gallon	25	25	32	79
		50	25	35	-
Column of 10 g. carbon	1 gallon @ 1 gal./hr.	25	25	33	49
		25	50	32	51
		50	25	36	69
		50	50	35	56
		500	25	19	58
		500	50	18	43

Malathion analysis:--A method for the determination of microgram quantities of malathion has been investigated briefly. The method involves the formation of acethydroxamic acid by reaction with alkaline hydroxylamine and subsequent formation of a colored complex with ferric iron at acid pH. The reaction appears specific for esterified carboxyl groups - several organophosphorus compounds not possessing such groups did not react similarly when tested. A manuscript describing the procedure has been submitted for approval.

Biological assay

A Drosophila colony was established this year at Denver. Addition of this index organism supplements the housefly and cucumber seed methods for residue analysis. Our work with Drosophila indicates some steps in cleanup procedures can be by-passed, and in some instances Drosophila provides the most sensitive method of analysis. Experimental techniques of housefly and seed assay were modified and refined. Seed analysis of herbicides in water has proved satisfactory in recent months.

Toxicant tolerance

Three chemicals being used or proposed for use in the fire ant control program were tested for their toxicity to redear sunfish. The chemicals were heptachlor and Allied Chemical's kepone and Compound GC-1283. Preliminary tests with Compound GC-1283, only recently considered for use in the fire ant program, indicated the compound to be relatively non-toxic to fish. Ten parts per million produced no mortality or adverse effects during a 10-day exposure period. The relative toxicities of kepone and heptachlor are presented in figure 2. Table 7 contains additional data on the toxicity of kepone in relation to time and temperature. Changes in both time and temperature had considerably more effect on the toxicity of kepone than on heptachlor.

Table 7:--Toxicity of kepone to redear sunfish. Median Effective Concentration, EC_{50}, 1/expressed in parts per billion active ingredient

Hours exposed	Temperature °F				
	45	55	65	75	85
6	-	-	-	1300	680
12	-	-	700	500	230
24	620	540	340	240	120
48	270	210	130	90	51
72	150	120	90	58	37
96	130	96	64	44	29

1/ Derived by Litchfield and Wilcoxon's method for analysis of dose-effect experiments. It is the estimated concentration that will produce 50 percent mortality. Each EC_{50} value reflects the results from tests at 4 or 5 different concentrations with 20 fish at each concentration.

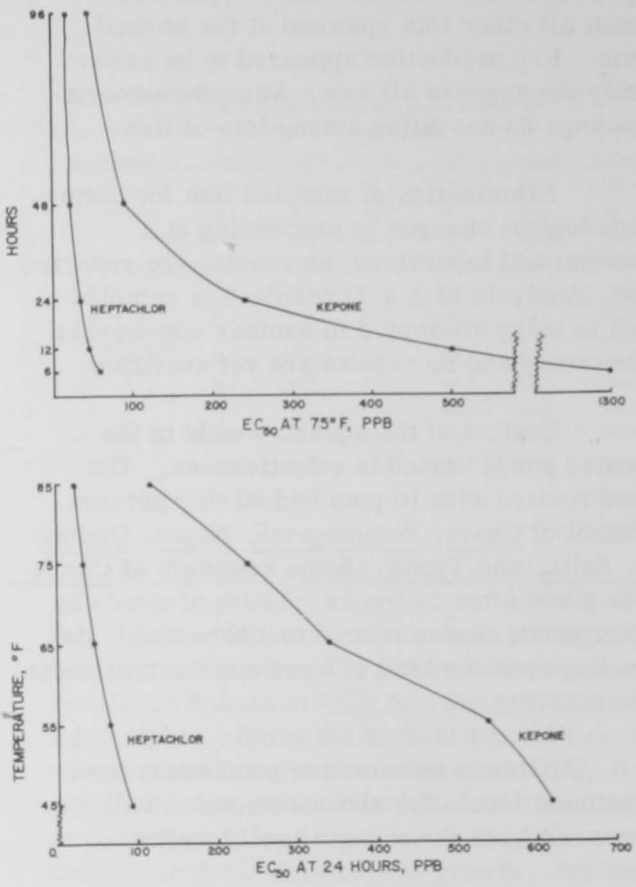


Figure 2:--The toxicity of heptachlor and kepone to redear sunfish at different temperatures and exposure periods. Median Effective Concentration (EC_{50}) expressed in parts per billion (ppb) active ingredient.

With both heptachlor and kepone the range of concentrations producing no effect and 100 percent mortality was quite small. In general, the concentration that killed all the fish was only 2-2.5 times larger than the concentration that produced no mortality and little effect.

Plastic barriers

A series of tests with several herbicides and polyethylene film demonstrated that herbicides will diffuse through polyethylene in aquatic situations. Similar tests with polyvinyl chloride established it as an effective barrier for the herbicide used. A short manuscript is being prepared on this subject.

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