PROGRESS IN SPORT FISHERY RESEARCH 1962

UNITED STATES DEPARTMENT OF THE INTERIOR
Fish and Wildlife Service
Bureau of Sport Fisheries and Wildlife

Circular 160

The Department of the Interior, created in 1849, is our Nation's Department of Natural Resources, concerned with management, conservation, and development of water, wildlife, fish, mineral, forest, and park and recreational resources. It also has major responsibilities for Indian and Territorial affairs.

As America's principal conservation agency, the Department works to assure that nonrenewable resources are developed and used wisely, that park and recreational resources are conserved for the future, and that renewable resources make their full contribution to the progress, prosperity, and security of the United States, now and in the future.

UNITED STATES DEPARTMENT OF THE INTERIOR
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Clarence F. Pautzke, Commissioner
BUREAU OF SPORT FISHERIES AND WILDLIFE
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Raymond E. Johnson, Chief

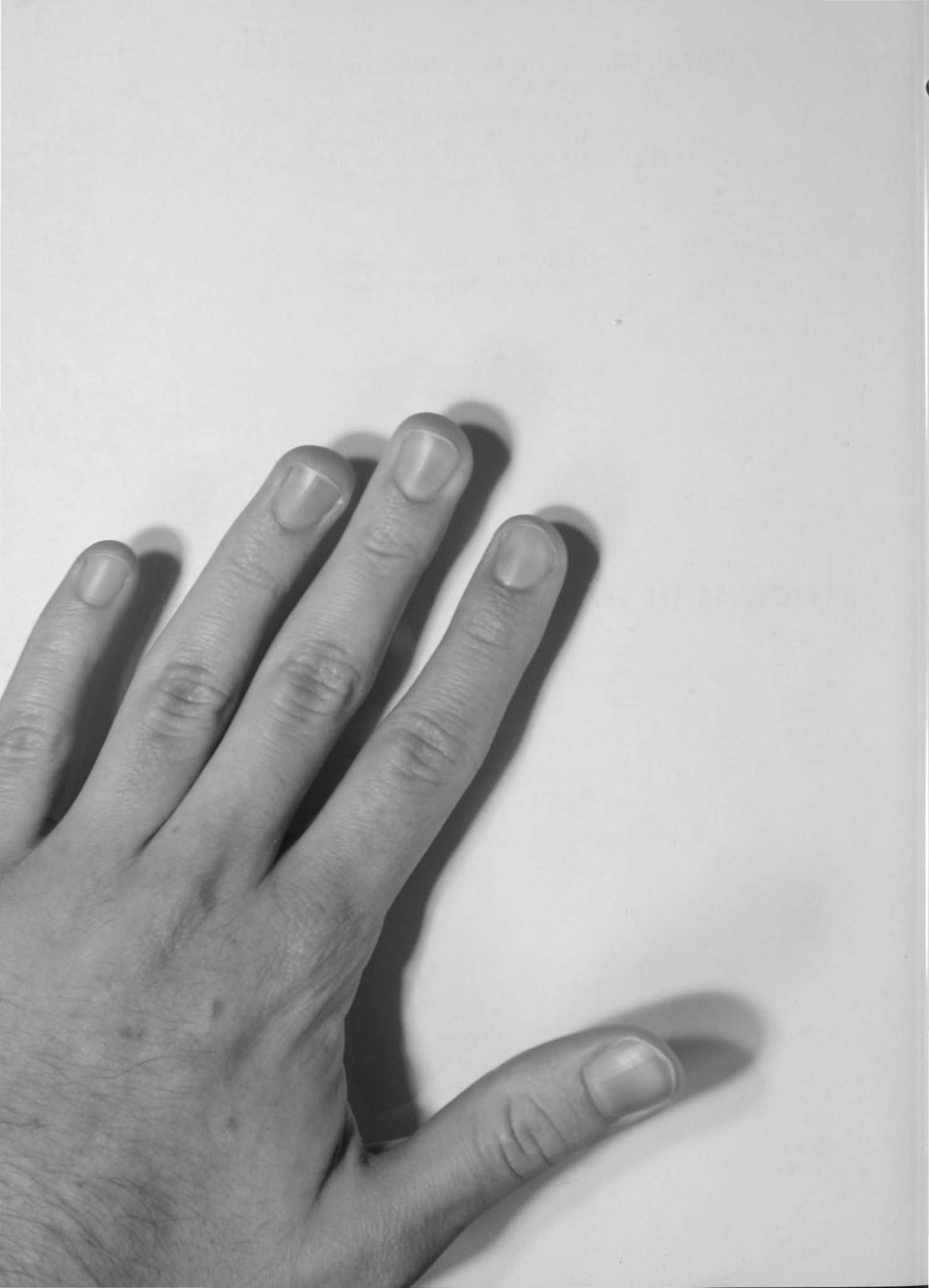
BRANCH OF FISHERY RESEARCH
Paul E. Thompson, Chief

PROGRESS IN SPORT FISHERY RESEARCH, 1962

Compiled in
BRANCH OF FISHERY RESEARCH

Fish and Wildlife Circular 160

Washington : March 1963



CONTENTS

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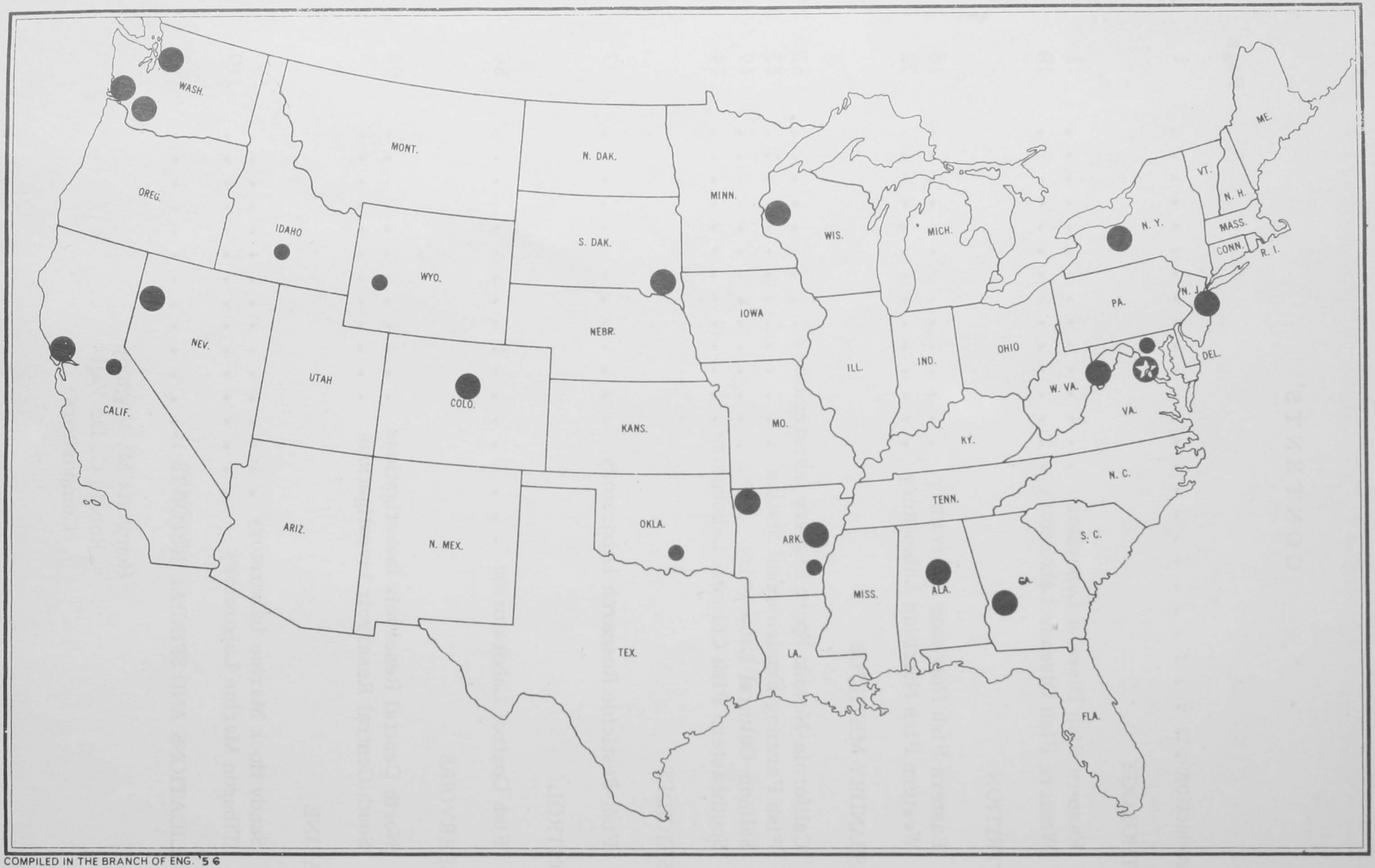

COMPILED IN THE BRANCH OF ENG. '56

SPORT FISHERY RESEARCH LABORATORIES AND STATIONS

PROGRESS IN SPORT FISHERY RESEARCH, 1962

INTRODUCTION

Continued progress toward the more productive and efficient research program needed to guide effective sport fishery management is reflected, we believe, in this report. Many research work units were successfully completed, each a step towards better understanding; more have been started; still others are scheduled, awaiting the availability of manpower and facilities.

Growth in research accomplishment is evident in the number of publications listed in this report, nearly 11 percent more than last year. Expanding research potential, a result of the appropriation of increased funds, is apparent in reported new activities, new facilities under construction, new laboratory equipment, and particularly in the year's 25 percent increase in total research staff.

In October, both the Fish Farming Experimental Station at Stuttgart, Arkansas, and the Fish Control Laboratory at La Crosse, Wisconsin, were officially dedicated to research activities already initiated. Construction was started on a new Fish Control Station at Warm Springs, Georgia. Another highlight of the year, and promise for the future, was the establishment at Fayetteville, Arkansas, of the new South Central Reservoir Investigations.

Mid-year appropriations, for fiscal year 1963, provided generously increased support of established research operations. The research program increase of $428,000 (F.Y. 1962, $1,449,000; F.Y. 1963, $1,877,000) includes: $193,000 for budgeted expansion of disease, pesticide, and marine studies; $45,000 to meet Pay Act costs; $105,000 for fish control research; and $85,000 for reservoir studies. Substantial funds were appropriated, too, for new and improved research physical plant. Included in the $790,000 of construction funds are: $320,000 for ponds and structures for fish-farming experimentation in Arkansas; $80,000 for steel prefab laboratory addition and quarters at the Convict Creek Experiment Station; $50,000 for site selection and planning for a major new laboratory to serve as headquarters for the growing investigations of the effects of pesticides; and $340,000 to purchase the selected Wyoming site for proposed new studies of trout genetics and selective breeding.

In this report, a listing of personnel concludes the section for each laboratory. To complete the Branch roster, personnel of the Washington, D.C., office are listed below. These people have done no research; they hope they have contributed to the year's progress by facilitating the capable and industrious efforts of a fine field staff.

Mr. Paul E. Thompson, Chief
Mr. Bruno vonLimbach, Assistant Chief
* Mr. Albert H. Swartz, Assistant Chief
Mrs. Henrietta M. Mugmon, Administrative Officer
Mr. Ronald C. De Vall, Secretary
Mrs. Nettie G. Bretzfelder, Clerk

* Transferred to Bureau of Outdoor Recreation October 13, 1962 and replaced by Mr. Robert M. Jenkins February 4, 1963.

PATHOLOGY

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

Detailed morphological and biochemical characteristics of cultures of Pseudomonas spp. which were isolated from fishes and kept at the culture collection of this laboratory were determined.

The effect of levan on the virulence and pathogenicity of selected strains of Pseudomonas spp. for rainbow trout was determined.

IPN virus was found to be associated with eggs and the egg fluid of normal appearing adult brook trout. The egg is the most likely means whereby this disease is transmitted.

A serological test for diagnosis of IPN disease has been developed.

Myxosoma sp. was found in the cartilage of bluegill at Leetown and the developmental stages are being described. Myxosporidia were found in Cottus and Catostomus at Lamar and, although similar to M. cerebralis, they were apparently different species.

Three new species of Gyrodactylus are being described.

Several chemicals were tested in an effort to find better compounds for prolonged-indefinite parasite treatment of fish in aquaria and troughs. Vioform^{R} and Betadine^{R} were the most promising.

Rainbow trout involved in hatchery feeding trials and maintained on one of the commercial pelleted diets have suffered high mortalities as well as anemia and lipoid degeneration of the liver.

Work on stored liver glycogen determinations on rainbow trout involved in the feeding trials is in progress.

The fishery research conference of the Bureau was held at Leetown for 3 days.

FISH PATHOGENIC BACTERIA

Antimetabolites

It is known that a combination of purine and pyrimidine antimetabolites and sulfonamides is more effective in arresting a bacterial infection in mice than either of the chemicals alone. Therefore, it was decided to see if a similar action could be demonstrated against fish pathogenic and related bacteria. Forty-eight antimetabolites were tested for in-vitro inhibition of Aeromonas salmonicida, A. liquefaciens, and Escherichia coli. Those antimetabolites which showed inhibition were tested in combination with sulfisoxasole (a sulfonamide drug) in an effort to demonstrate a synergistic effect. The tube dilution method of testing was employed using the above bacteria. No clear cut synergistic effect was found in preliminary tests. Some difficulty was encountered in dissolving some of the antimetabolites in either distilled water or 95 percent alcohol and it is possible that this prevented a potential synergistic effect. However, since the results were not promising, the project was temporarily discontinued.

Pseudomonas organisms from fish

Because Pseudomonas spp. produce bacteriemic infections among freshwater fishes, especially pondfishes, it seemed appropriate to examine characteristics of such organisms isolated from fish. Also, the ability of these bacteria to produce disease in hatchery reared trout was considered an important factor and was examined. From the morphological and biochemical tests performed with these bacteria, it was possible to define this type of organism, at least as far as the genus. An organism is considered a member of the genus Pseudomonas if it is a gram negative, polar flagellated rod, oxidative in its carbohydrate metabolism, and producing cytochrome oxidase. The ability to produce fluorescent compounds is also a very important characteristic when present. However, since choice of medium is important, a medium especially designed to detect fluorescent compounds should be used. On the basis of our experiments it was neither possible nor

desirable to assign species to these fish-isolated pseudomonads. However, by comparing our data to published results, it was possible to narrow taxonomic position to species by using temperature relationships and proteolytic activity. Most of the organisms studied were thus identified as Pseudomonas fluorescens, while the remainder were P. putida or P. fragi.

It was found that some of the strains produced a high molecular weight polysaccharide called levan. Since we thought that the presence of levan might increase the pathogenicity of these strains for hatchery-reared rainbow trout by protecting bacteria from phagocytosis, these strains were used in the pathogenicity studies. Consistently higher mortality of the hatchery rainbow trout occurred when they were injected with cells plus levan than without. Levan injected intravenously or intraperitoneally does increase the pathogenicity of certain bacteria, but not by physically enveloping the bacteria as was suspected, but rather by blocking the immune response (Shilo, 1962). The molecular size and amount given markedly influences the ability of levan to enhance infection. Higher mortality occurred when levan was present with the cells, however, the extent of the difference varied among the strains used. Since neither the purity, nor amount of the levan given to the fish was known, it is possible that these factors were responsible for the variation. Nevertheless statistical analysis indicated that the results obtained are highly significant.

Actinomycete-like cultures

Two actinomycete-like cultures were isolated from diseased fish. One was isolated from rainbow trout fingerling from Bowden Spring National Fish Hatchery and the other from golden shiner from the Fish Farming Experimental Station, Stuttgart, Arkansas. Both cultures have been purified, and a preliminary test of pathogenicity was performed with the Bowden Spring culture.

Corynebacterial kidney disease

Reports on the occurrence of kidney disease have accumulated in the files of the laboratory and of the fish hatchery biologist and a pattern in the occurrences gradually unfolded. The stations with the greatest incidence of this disease had soft water supplied from a stream. In contrast, stations with very hard water seldom have any kidney disease. James Warren, one of the trainees, made a broad survey of this relationship and showed that there was a highly significant and consistent relationship between the mineral content of water and the occurrence of kidney disease. Based in part upon chemical analyses which he made and upon analyses furnished by others, Mr. Warren found graded increases in kidney disease mortality among stations having progressively softer waters. In some of the eastern trout hatcheries the incidence of external parasites was high when the water was soft and was taken from an open stream.

Erythromycin is the drug of choice in the treatment of kidney disease, but erythromycin thiocyanate has been employed as a substitute drug. One report of treatment with erythromycin thiocyanate stated that rainbow trout suffered rather severe toxic reactions during treatment. Mr. Warren employed the same strain of trout in toxicity trials at the laboratory and found that five times the recommended dosage level was necessary to evoke overt symptoms of toxicity. Concurrent administration of iodine with erythromycin thiocyanate did not prevent symptoms of toxicity.

Manufacturers have recently adjusted the prices of the various forms of this antibiotic so there no longer remains justification for using the thiocyanate.

VIRAL DISEASES

Infectious pancreatic necrosis

Infectious pancreatic necrosis (IPN), chinook salmon, and sockeye salmon virus diseases are three viral infections recognized at present as being causes of severe mortality among salmonid fishes propagated in North America. The species, age, and geographic restrictions of the two diseases of Pacific salmon are of signal importance in diagnostic work. IPN, however, has fewer restrictions in its occurrence, and histologic techniques have been the standard for diagnosis. Whereas histologic examination can show the pathology

of pancreatic necrosis, it cannot identify the cause, and more than one agent can attack the pancreas. Serological methods permit rapid and accurate identification of pathogens, and the methods have been applied to identification of IPN virus.

Bacteria-free filtrates used in these studies were prepared from homogenates of suspect material and divided into three portions. One portion was inoculated into susceptible cells such as RTG-2 and into refractory cells such as FT cells. A second portion of the filtrate was heat treated (to inactivate virus) and a third portion was mixed with immune serum (anti-IPN) of known titer prior to inoculation of susceptible cells. Positive results were usually obtained within a day and a half and often in less than 24 hours. If IPN virus was present, cultures inoculated with untreated filtrate showed rapid cytopathic change, but cultures inoculated with heat-treated filtrate and with filtrate which has been neutralized with immune serum were unaffected. As yet unnamed viruses may also produce cytopathic changes in RTG-2 cells, but it is not likely that such viruses will be neutralized with anti-IPN serum.

IPN has long been considered to be transmitted with or by trout eggs, and such transmission was established as fact. The filterable agents which were isolated from egg fluids of brood fish at a suspect source were fed to susceptible brook trout. Inoculated lots of brook trout developed IPN after the usual minimal incubation period, and the mortality was nearly complete (98 percent). Control lots did not develop IPN.

Using RTG-2 cells incubated at 20° C., egg fluids were titrated to determine the amount of virus they contained, and sera from the same fish were titrated against a standard dose of virus in order to measure the antibody level. End points were determined by the method of Kärbar or by the Reed and Muench method. A summary of the results is presented in table 1.

Large amounts of virus were found in egg fluids from one-fourth of the fish, but there was no measurable antibody in blood serum from the same individuals. In contrast, as tested against 68 cell culture infective doses 50 percent (ID_{50}) high neutralizing titers were found in sera of one-fourth of the fish, and low levels were found in two other sera. There was a coincident low level or absence of virus in the egg fluids from the fish with measurable antibody. Two fish had neither virus nor appreciable antibody levels. A single incidence of viremia occurred in the fish with the greatest amount of virus in the egg fluid. Confirmation of this result was sought in a second test but was not obtained.

Eggs were washed twice in a physiological saline and homogenized. The virus titers in egg homogenates were considerably lower than the titers of the corresponding egg fluids. Having passed IPN virus through 50 mμ filters and considering the pore size of the average trout egg chorion (1000 mμ) it seems almost inescapable that some virus would be within or beneath the chorion. Whether or not the virus was within the egg was not determined in this work.

Virus titrations were performed with and without egg homogenates being present. There was no loss of virus in the titration performed with egg homogenate, therefore, added validity was obtained for the results presented.

Eggs, egg fluids, sperm, spermatic fluids, and blood sera have been obtained from additional fish for further study. The results already reported however, do provide guidelines for selecting female brood stock which are likely to be free of the infection, and such selection could provide a nucleus of brood stock and implement a rational means of controlling the disease.

Lymphocystis disease

At some hatcheries lymphocystis disease occurs infrequently and in small numbers among presumably susceptible populations, but at other hatcheries the disease is common and appears to be highly infectious. The reasons for this difference are unknown, but it has been speculated that ectoparasites or

Table 1:--Serum neutralization titers and the occurrence of virus in sera, eggs, and egg fluids of Bellefonte strain brook trout (Salvelinus fontinalis)

	Fish Number											
	1	2	3	4	5	6	7	8	9	10	11	12
Neutralization titer of serum against 68 ID_{50} IPN virus	0	1:1024	1:2	no serum	1:512	less than 1:16 *	less than 1:16 *	1:512	0	0	no serum	1:32
Titer of virus (in ID_{50}/ml) in egg fluid after 10 months at -20°	$10^{6.25}$	0	0	0	0	0	0	0	10^{0}	$10^{6.5}$	$10^{4.75}$	0
Titer of virus (in ID_{50}/ml) in egg homogenate	$10^{1.0}$	0	0	0	0	0	0	0	0	$10^{1.75}$	$10^{0.625}$	0

* Volume of serum not sufficient to make dilutions less than 1:16.

water chemistry may be involved. Effects of soft water and of certain ectoparasites upon infectivity of lymphocystis disease were investigated. Since there is no known treatment, therapy with chloramphenicol was also investigated.

Following 10 months storage at 4° C., desiccated preparations of virus were tested for viability and were found to be infectious. The virus readily passed through a 300 mμ membrane filter, but it was retained by a membrane with an average pore size of 100 mμ. This size determination agrees with measurements made by other means.

Chloramphenicol administered at the rate of 65 mg/kg/48 hours before subcutaneous injection of virus and every 48 hours for 18 days thereafter did not prevent infection, but there was delayed appearance of secondary sites of infection in fish so treated. Monogenetic trematodes were the only ectoparasites found to persist through the period of experimentation, and they failed to appreciably reinfect a portion of the fish from which they had been removed prior to the start of the experiment. Resistivity of the water was maintained at an average of 39,500 ohms. Previous work was done in water having less than 2,000 ohms resistivity. Inoculated fish were present at a ratio of two to each uninoculated fish, but only one of the latter contracted the infection. It was concluded, therefore, that in this work neither soft water nor monogenetic trematodes effected fish-to-fish transmission of the disease. There was, however, a twofold greater incidence of secondary infection sites than had been obtained in previous work with the same strain of fish held at the same temperature but in hard water and after treatment to remove ectoparasites.

In a joint study with Dr. Roland Walker, Renssalaer Polytechnic Institute, Troy, New York, parallel studies with the electron microscope and light microscope have been undertaken on known-age lymphocystis tumors. The tumors were experimentally started (fig. 1) in a group of bluegill at the laboratory. The light microscopy has been nearly completed, and 24-hour lymphocystis cells have been found. This is the youngest lymphocystis cell yet described.

Additional efforts to cultivate the lymphocystis cell in vitro have been either negative or equivocal. Tissue containing

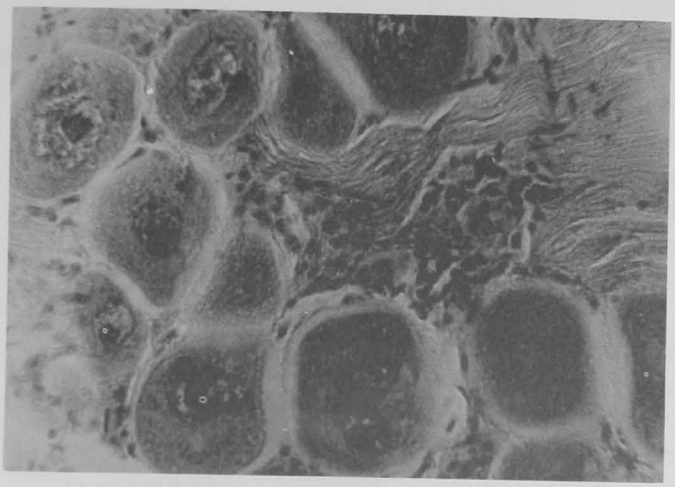

Figure 1:--Thirteen-day-old lymphocystis cells from experimentally infected bluegill (Lepomis macrochirus). Cellular enlargement is already advanced, and inclusions are being formed.

3-day-old lymphocystis cells was excised and placed in culture at 25° C. Three weeks later the material was examined by histologic section, but there had been little or no additional development of the infected cells.

Infectious dropsy

Goldfish with symptoms of infectious dropsy were submitted for diagnostic work. Since Russian investigators consider this to be a viral infection, peritoneal fluid was aseptically aspirated, diluted, filtered and inoculated into both RTG-2 cells and FHM cells. Filtrates proved to be free of bacteria and neither cell type showed viral effects.

FISH TISSUE CULTURE

Cell and tissue culture

RTF-1 and RTG-2 cell lines of rainbow trout origin are now 3 years old, and the FT line of frog origin is 1-1/2 year old. These lines were established at the laboratory, and are finding increasing application in fish disease research and in other research laboratories in the United States, Argentina, and Germany.

The FHM line of fathead minnow origin has been obtained from Dr. R. G. Malsberger, Lehigh University, in whose laboratory it was isolated. Maintenance and use of this line increases the likelihood of identifying new pathogens in diagnostic work.

Preliminary work with the newly acquired Revco ultra low temperature cabinet has shown that properly prepared RTG-2 cells, like established cell lines from other animals, can be frozen and held at -87° C., with maintenance of their viability. This method has permitted storage and survival of mammalian cells for several years, and it is expected that fish cells will be equally amenable to prolonged storage.

FISH PARASITOLOGY

Myxosoma cerebralis

Whirling disease caused by Myxosoma cerebralis has spread to a third location in the State of Pennsylvania. We believe, however, that it is under control but not eradicated at the Connecticut hatchery. If this disease continues to spread, as it has in the past 4 years, it will probably affect most trout and salmon hatcheries which use either earthen ponds or stream water. Life history and pathology studies are being continued on a closely related species of Myxosoma present in the cartilage of bluegill at Leetown.

Myxosoma sp. of bluegill cartilage

Shortly after hatching, bluegill from contaminated ponds were collected and kept in aquaria in order to study the development of the Myxosoma and the resultant histopathology. Small trophozoites were detected at about 2-3 weeks post-infection (fig. 2) and the first spores were seen in the 6-8 weeks old infections. In general in Myxosoma infections the maximum spore production is reached at about 6 months after which the production of spores declines, when the lesions in the gill skeleton open up and the spores are released into the water. Following this, very few spores can be found in the fish.

Attempts to infect bluegill experimentally with this Myxosoma were negative as

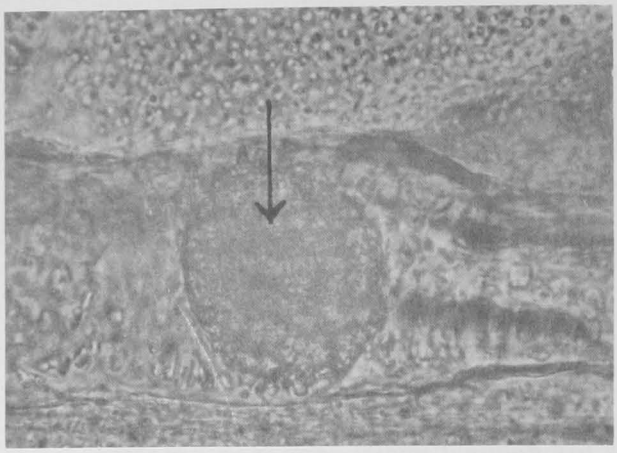

Figure 2:--Trophozoites of Myxosoma sp. in cartilage of small bluegill.

were earlier attempts to infect trout with Myxosoma cerebralis. Spores were fed to uninfected bluegills 2-3 weeks old and were recovered intact from the feces. The polar filaments were not extruded and the infective sporoplasms were still in the spores. For some time it has been suspected that representatives of zooplankton are responsible for carrying Myxosoma spores to fry. Therefore, spores were fed to laboratory reared copepods and were recovered from their intestines. Many polar filaments were extruded, indicating biological or chemical stimulation. There is a possibility that they are either necessary or suitable as transport hosts. This Myxosoma of the bluegill appears to be very closely related but not identical to that occurring in the salmonids. It has been found at Leetown in bluegill, green sunfish, and largemouth black bass, but not in cyprinids or salmonids.

Other Myxosporidia

The coelozoic myxosporidia, Chloromyxum, Mitraspora, Myxidium, Myxoboliatus, and Wardia are also present in the Leetown bluegill. A report on the histopathology caused by these gall bladder and kidney tubule parasites will be forthcoming when histopathologic studies have been completed. The bladders (fig. 3) and tubules were sometimes heavily parasitized and some trophozoites have been seen in adjacent non-coelozoic tissue. It is planned to describe these species of Myxosporidia from the bluegill. Alexis Knight of the Lamar National Fish Hatchery has been making a survey of myxosporidia in fish other than trout from their water supply in an attempt to determine the reservoir of whirling disease. He found spores in Cottus and Catostomus which resembled Myxosoma cerebralis but proved to be different species.

Preserved gizzard shad collected from an epizootic at Buckeye Lake, Ohio, contained huge visceral cysts of a microsporidian which apparently killed the fish. Robert Putz has prepared a manuscript on this new species which will be named Plistophora cepediani. The donator of the material is interested in the possibility of using this parasite for shad control. To date we have no records of its being found in any other fish of that lake or elsewhere and therefore it might be host specific enough for use in biological control.

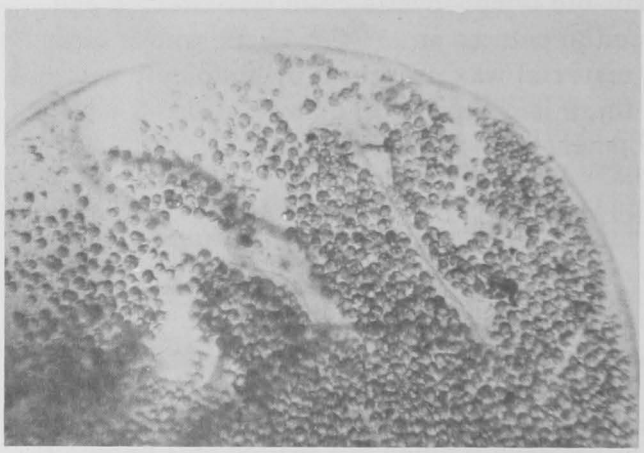

Figure 3:--Many trophozoites of Chloromyxum sp. in the gall bladder of small bluegill.

Epistylis and Scyphidia

These parasites have been and are being collected and studied from hatchery and wild fish. It is planned to compare them later. To date it appears that there are several species which parasitize rainbow, brook, brown, and cutthroat trouts, perch, catfish, and Atlantic salmon. Living material was obtained from Semotilus and Lepomis held at 54° F. in the hatchery.

Trichophrya

Material was submitted from brook and rainbow trout, Atlantic salmon, perch, bluegill, catfish, goldfish and largemouth black bass to Dr. R. W. Hull, Northwestern University, who has published a revision of the genus (Culbertson, J. R. and R. W. Hull, 1962. J. Protozool. 9:455-458).

Gyrodactylus of bluegills

This parasite is being maintained on small bluegill in the laboratory. Howard M. Jackson, 1961-62 trainee, determined that this Gyrodactylus is more easily transmitted to non-infected fish by skin scrapings than by live infected fish in the same aquarium. Non-infected fish suspended in baskets in the trough containing infected fish likewise became infected, but those in baskets in contact with the bottom became more heavily parasitized.

New species of Gyrodactylus

Robert Putz has prepared a manuscript on Gyrodactylus atratuli n. sp. from Rhinichthys atratulus and G. margaritae n. sp. from Semotilus margarita along with an illustrated synopsis of the genus. A report on G. macrochiri n. sp. has been prepared. Other species of Gyrodactylus are still being submitted to Dr. G. Malmberg of Sweden who plans to prepare a monograph of the genus.

Goldfish parasites

In addition to a bacterial infection caused by Aeromonas liquefaciens the following parasites were found in a lot of diseased goldfish: Chilodonella, Dactylogyrus, Eimeria (Coceidia), Gyrodactylus, Hexamita (Urophagus?), Ichthyhopthirius, Mitraspora, Scyphidia and Trichodina. At 40-54° F. Chilodonella and Gyrodactylus were very numerous but Ichthyopthirius flourished at 66-69° F. The Chilodonella probably contributed heavily towards the mortality, but most surprising was the presence of an enteritis producing Eimeria. Long, opaque, white fecal casts resembling tapeworms, consisting of mucus and spores, were seen trailing the fish. The entire intestine was parasitemic and this parasite probably contributed greatly to the mortalities. Eimeria has been reported from North American freshwater fish only twice; both times from wild brook trout. Attempts are being made to maintain this interesting parasite in the laboratory.

Bolbophorus confusus

The metacercaria of this parasite was identified at this laboratory for Mr. A. C. Fox who has published an account of its incidence in trout from Montana. This is the first North American record of the metaceracria. (Fox, A.C., 1962, Trans. Amer. Microsc. Soc. 81:179-184).

Lernaea cyprinaceae

This parasite is being collected with the hopes of publishing a note on its spread around the world with fish culture. The most recent specimens are from frog tadpoles collected by Mr. Jimmy Camper, Pisgah Forest National Fish Hatchery. This is one of two parasites that fish have in common with frogs.

Fish parasite treatment

In an attempt to find better compounds for prolonged-indefinite treatment in aquaria and troughs some preliminary tests were made using yearling bluegill infected with Gyrodactylus macrochiri at 54° F. Various concentrations of Betadine (polyvinylpyrolidone-iodine complex), potassium permanganate, Roccal, Triburon (Hoffman-LaRoche Ro 5-0810/1) and Vioform (2 chloro-7-iodo-8-hydroxyquinoline) were tested. Betadine and Vioform should be tested further and potassium permanganate at 2 ppm has been recommended for pond treatment, but may be toxic in clean aquaria.

Miscellaneous parasitology

The gills of striped bass mortalities from Beaufort, North Carolina were literally smothered with the larval forms of Ergaislus labracis, a common copepod of that fish.

Dr. Cope submitted some moribund Lepomis microlophus which had large masses of Spinitectus (Nematoda) in the intestine. Similarly infected bluegills were collected from farm ponds of this area, and it is suspected that heavy infections do cause disease and mortalities. The worms have been observed migrating out of heavily infected fish. Many eggs become trapped between the gill lamellae (fig. 4). It is not understood how the eggs get between the lamellae but we presume that they are deposited by migrating worms.

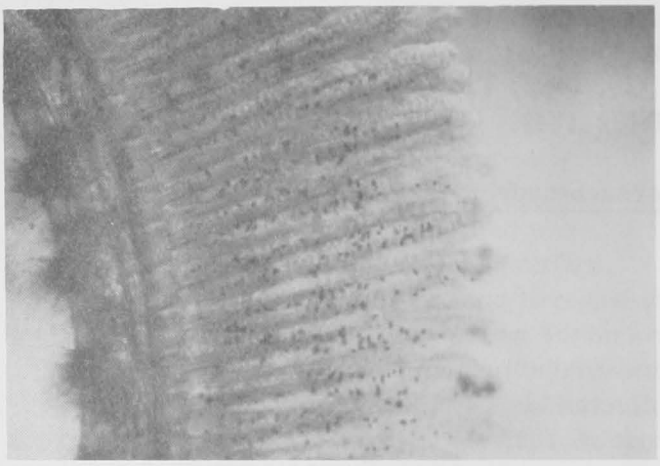

Figure 4:--Eggs of Spinitectus sp. in gill of small bluegill. The numerous dark-appearing objects are eggs.

Wardia (Myxosporidia) which has been recorded only once (ovary of Lepomis humilis) has been found in bluegill kidney.

Mr. Paul Osborn, Ozark Fisheries, submitted some nematodes from the caudal fin of goldfish which proved to be Philometra carassii (Nematoda:Filaroidea) recorded only from Japan and Europe.

Dr. Cable, Purdue University, gave us a fine specimen of catfish ovary infected with Acetodextra ameuri which are grossly visible as brown spots. This interesting trematode continues its life cycle by a very dramatic procedure. The worm is expelled during spawning and the eggs explode out of the uterus in a few seconds due to hypotonicity.

Roccal was found to kill Gyrodactylus and Ichthyopthirius in 20 minutes at a concentration of 200 ppm when used as an equipment disinfectant.

HISTOPATHOLOGY

In 1962, 130 cases were processed and examined histopathologically by this laboratory. Several of these cases consist of specimens from the Leetown research program. The major projects being, a study of the development of the lymphocystis cells in bluegill, host response to parasite infections, and the transplantibility of hepatoma into the liver of healthy rainbow trout. The results of these examinations are described in proper sections of this report.

Requests from both State and Federal hatcheries in nine States were received for pathological examination of diseased fish by the hatchery biologist. Many of the requests concerned large populations suffering a high mortality rate. The histological and hematological examination often revealed the presence of hepatoma, lipoid degeneration of the liver and acute anemia. Hepatoma was confirmed for the first time in 2- and 3-year-old rainbow trout in Virginia and West Virginia hatcheries. Preliminary surveys indicate that the incidence of hepatoma in these fish is high.

Lipoid degeneration of the liver in rainbow trout

The Missouri Conservation Commission, in cooperation with this laboratory, made an attempt to determine the cause of a severe outbreak of lipoid degeneration of the liver in rainbow trout. The fish developed this condition while being fed a commercial pelleted diet. When the diet was changed to 100 percent beef liver or mixed 50 percent beef liver and 50 percent pellets a marked improvement occurred.

NON-INFECTIOUS DISEASES

The feeding trials at selected National hatcheries were carried out for the second year as one of the approaches to the study on the relationship between nutrition and the incidence of hepatoma in rainbow trout. Trout used in this experiment at the Leetown hatchery are

being fed three commercial pelleted diets and a "meat" diet consisting of beef liver and spleen. Blood examination was carried out periodically during 1962 on 10 trout selected at random from each test diet. The most important differences between trout fed different diets were in hematocrits and hemoglobin (table 2). Trout fed commercial diets B, C, and the meat diet appear normal. In April trout fed the commercial diet A started to show signs of anemia and lipoid liver degeneration which is becoming progressively more pronounced. During the fall months mortalities were rapidly increasing in trout fed the commercial diet A. As it was feared that all would die leaving none for hepatoma observation, the remaining trout on diet A were divided into two equal groups November 8. One group was continued on diet A and the other was fed a mixture consisting of half diet A and half meat. As of December 23, there was a 51.5 percent loss in fish fed diet A and 38.4 percent on diet A plus meat. All dead fish were autopsied and showed yellow livers and intestinal tracts without food but containing mucus colored with bile.

Table 2:--Average hematocrit and hemoglobin levels in 2-year-old rainbow trout (Soap Lake, Washington), fed 4 different diets. Averages were calculated from 10 fish per diet.

Date of Examination	Commercial Diets A		B		C		Meat Diet	
	Ht	Hb	Ht	Hb	Ht	Hb	Ht	Hb
Jan. 23	42	8.4	42	8.6	45	9.3	39	8.7
Feb. 20	38	7.6	43	10	45	9.6	39	8.0
Mar. 27	40	7.4	41	8.3	41	8.5	41	8.2
Apr. 23	32	7.0	43	9.0	42	9.3	39	7.9
Jun. 13	37	7.4	41	9.6	42	8.7	37	9.3
Jul. 30	30	7.4	38	8.9	38	9.8	34	9.2
Aug. 20	30	7.5	39	9.7	39	9.4	36	9.2
Oct. 8	26	5.8	44	10.1	49	10.4	40	9.7
Dec. 12			38	9.4	37	8.7	41	10.1

No hepatoma was seen in rainbow trout fed any of the test diets at Leetown. The other parameters which are being determined and recorded for all groups are length, weight, weight of liver, and the refractive index of plasma collected from hematocrit capillaries.

GENERAL

Tyrosine crystals in furunculosis agar

Recently workers from the Seattle Laboratory indicated that crystal formation in commercial Furunculosis Agar was causing confusion, because the crystals have the appearance of small bacterial colonies. The crystals were suspected of being tyrosine.

Glassware washer

The purchase and installation of a Heinicke HW 5000 glassware washer has permitted a rapid expansion of cell culture production. Manual preparation of glassware which required 8 or more hours to complete can now be processed in slightly more than one hour. This has made possible the routine planting of 400 to 450 cultures per week. The washing time for bacteriological glassware has also been greatly reduced.

Epithelocystis

Histological examinations of small white spots on bluegills indicated a possible virus infection. If subsequent work supports the presence of virus, it is planned to name the condition epitheleocystis because of its slight resemblance to lymphocystis.

Toxicity

Howard Jackson determined the lethal concentrations of formalin, Roccal, and acriflavine on bluegills at 54° F. He plans to report this work later.

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

Highlight items of 1962 research activity were as follows:

Immunopathology program activated.

Tissue culture techniques established as a routine, diagnostic practice.

Infectious pancreatic necrosis confirmed in rainbow, brook, and cutthroat trout in western States.

Relationship between commercial dry diets and hepatoma established.

Significance of Nanophyetus salmincola in salmon habitats established.

IMMUNOPATHOLOGY

In January of this year, an immunopathology section was added to the Western Fish Disease Laboratory. The coordinator of this section is a senior in the College of Veterinary Medicine, Washington State University, and also a holder of degrees in microbiology and immunology.

The major functions of the immunopathology section are: (1) to determine the fish host response to infection by disease producing agents; (2) to provide serological means of identifying fish pathogens in vivo and in vitro; (3) to describe the cellular site(s) and nature of the immune response in salmonids; (4) to provide a means of immunological prophylaxis in fish against disease producing agents.

This section made four significant contributions this past year to the program carried on at the Western Fish Disease Laboratory:

1. A survey of the literature pertaining to fish hematology, serology, pathology, and immunology. This is now complete to date.

2. The adaptation to fish of the technique of homologous and heterologous transplantation of tissues into the anterior chamber of the mammalian eye. We have maintained for three months, rainbow trout kidney (anterior portion), spleen, pancreas, and liver (both biopsy and tissue culture) in the anterior chamber of the eyes of adult albino rabbits and rainbow trout.

At 2-3 months, however, a great deal of degeneration occurred in the tissues transplanted into the rabbit eyes. The reasons why the rainbow trout tissues apparently should thrive for nearly three months in the anterior chamber of a rabbit's eye and then suddenly degenerate are currently being investigated. The transplanted tissue residing in the anterior chamber of the eye represents an isolated organ system fully capable of normal physiological function without being modified by or modifying the function of other organs in the host. This feature has been experimentally validated in mammals.

3. A means of orally immunizing rainbow trout against the etiologic agents of red mouth and furunculosis was investigated. The details of the study are presented in the Bacteriology section report.

4. The application of the technique of immunodiffusion to demonstrate the presence of circulating precipitating antibodies in rainbow trout was rewarding. To our knowledge there are not conclusive reports relating to the demonstration of the production of precipitating antibodies by cold-blooded vertebrates. The details of this are presented in the Bacteriology section report.

Collaborative work with the Fur Animal Disease Laboratory, USDA, Washington State University, on the etiologic agents and vectors involved in salmon poisoning disease of dogs was begun early in the year, discontinued for the summer months, and renewed this winter. Our interest lies in the possible pathologies that may or may not occur in fish due to the metacercariae and/or the rickettsia. Data obtained thus far indicate that the technique of immunodiffusion can detect the speficic antigens

of the various stages in the life cycle of the fluke, Nanopheytus salmonicola, in dog, snail, and fish tissues.

BACTERIOLOGY

An experiment was started to test the feasibility of oral immunization of rainbow trout against "redmouth" disease and furunculosis. Three vaccines were prepared consisting of pelletized food containing killed cells of the etiologic agents of "redmouth", furunculosis, and a combination of both. Each vaccine was fed to respective lots of 100 fish for six weeks at which time samples from each group, as well as controls, were challenged by inoculating homologous viable bacterial cells directly into the stomach. Inconclusive results were obtained from this challenge. Virulence of the stock "redmouth" strain used in the study was enhanced by repeated fish passage and a number of lyophilized cultures were prepared following determination of the $L.D._{50}$. This material was used for intraperitoneal challenge of 10 vaccinated and 10 non-vaccinated fish on three separate occasions. Of the 20 vaccinated fish used in the first two challenge experiments, 10 survived while there were only five survivors among the 20 non-vaccinated fish in the two groups. The presence of "redmouth" bacteria was confirmed in all mortalities by cultural methods. The third challenge was given late in the year, and at the present time, only one mortality has occurred, that being a non-vaccinated control. Based on the time interval between initiation of challenge and start of mortalities in the first two challenges, it appears however that control fish in the last challenge experiment are not responding as rapidly as anticipated. Studies on the vaccination against A. salmonicida have been delayed due to the lack of a suitable test culture.

The technique of immunodiffusion to demonstrate the presence of circulating precipitating antibodies in rainbow trout was applied using as immunizing agents four different soluble antigens prepared from an Aeromonas salmonicida culture. Early results were extremely rewarding as precipitating antibodies were demonstrable in each of the four groups of fish. It has recently been determined, however, that some non-specific reactions may be involved as positive reactions have been obtained from supposedly normal rainbow trout sera. Also, the fish from which the normal sera were taken may have been exposed to a low grade furunculosis infection during their earlier hatchery life. Present work is concerned with determining what factors may be involved.

Immune rabbit sera have been prepared from soluble antigens of eight Aeromonas cultures. This material is being used in comparative immunodiffusion studies to determine the value of this method in identifying and classifying members of this genus.

A fish kill at Black Lake, near Olympia, Washington, was investigated at the request of the State Game Department. Black crappie (Pomoxis nigromaculatus) was the species most significantly affected, although a few yellow perch and carp also were involved. Unfortunately the call for assistance was received at a time when only dead specimens were available. A rather unusual organism from the standpoint of fish pathology was isolated from the few fish suitable for culturing. This type of kill is said to be recurrent at Black Lake, and we plan to study the disease further in 1963.

To determine whether mycobacterial infections in fish may occur at time of fertilization, silver salmon eggs were exposed to a viable suspension of mycobacterial cells just prior to addition of milt. Survival to the eyed stage was almost 100 percent. White-spot disease became manifest at the time of hatching, and mortality was high. No evidence of acid-fast bacteria has been seen in any of the affected fish however. Mortalities among surviving fish will be examined for the presence of infection.

TISSUE CULTURE AND VIROLOGY

IPN was observed in and isolated from rainbow, brook, and cutthroat trout; the latter being a new host. This disease was confirmed in fish from Washington, Montana, Utah, and Nevada. Tissue culture techniques were advanced to a point where they were used routinely for the study of viruses, suspect virus diseases,

and the culturing of fastidious organisms. Three virus diseases of fish, IPN, SRCD, and OSD, were studied and some comparisons made as to filterability, pathology, and effect on tissue cultures. There are some similarities among the three viruses, but they are distinctly different entities.

The culturing of cells from tissues of salmonid fishes reached a high level of reproducibility. Primary cultures of organs, other than the liver, were prepared and utilized for the study of virus diseases as well as for the culture of fastidious organisms such as mycobacteria and the hemoflagellate Cryptobia. In addition we have cell lines from the Eastern Fish Disease Laboratory (rainbow trout gonad) and from the University of Miami (grunt fin). Our cell subcultures were a long way from being established as cell lines, but during this past year important progress was made toward achieving this goal.

Primary isolates of acid-fast bacilli were maintained through four bi-weekly subcultures. Within the first two subcultures there appeared to be an increase in the number of bacilli followed by a period during which individual cells maintained viability, but numbers decreased terminating in a state of nonviable organisms. Tissue culture fluid, agar media, and other artificial methods were negative from the start.

Maintenance of Cryptobia was also possible for short periods of time, but true propagation was not accomplished.

Although field studies of SRCD were complicated by "white-spot" troubles, data from the 1961-1962 investigation indicated that the disease could be controlled by manipulation of the fish's environment. Laboratory studies with additional agents for the chemical and/or biological control of the virus were negative. The agent was cultured on chinook fin tissue, a technique that should be of great value in future studies. Investigation of resident populations of fish in the Coleman area was initiated, but results were negative at the end of the year. Ovarian involvement for the transmission of the agent still appeared as the most feasible explanation at the close of 1962.

IPN was diagnosed and the virus isolated from five separate outbreaks in the West, and IPN was suspected in at least two other cases. With the exceptions of the Springville, Utah State (rainbow trout) and Federal (brook trout) hatcheries, all cases were linked directly or indirectly with cutthroat brood stock at Creston, Montana. Of great interest to our laboratory was the diagnosis, histological confirmation, and the isolation of the virus of IPN from 18-month-old 10-inch cutthroat trout; prior reports concerned only younger fish. Serological studies were initiated at the end of the year to determine whether all isolates are similar, or whether there are strain differences. Samples of all isolates, except that from Nevada, were sent to the Eastern Fish Disease Laboratory for comparison with their strains.

Some of the Creston cutthroat brood stock was transferred to Leavenworth National Fish Hatchery. A program was started to determine if IPN virus carriers could be detected and eliminated from this group of fish. Sixty fish, supposedly all females, were examined and, although some were shown to have IPN antibodies in their sera, none were proven to be carriers. This work will be continued.

PATHOLOGY

Histological evaluation of the liver samples from the second commercial dry diet experiment initiated last year at the Hagerman National Fish Hatchery by the Branch of Fish Hatcheries was terminated at the end of this year. Twenty liver tissues of fish on each of the seven diets were fixed in Bouin's. If extensive gross lesions were observed on any of the livers, not only the liver but the entire viscera including the liver, alimentary tract, spleen, kidney, and gills were fixed so that more thorough examinations could be made at the laboratory for metastasis. Monthly histological examinations were made until the third quarter at which time it became apparent that the occurrence of hepatoma had become fairly regular. It was then decided that the subsequent

samples would be processed and analyzed every other month unless otherwise indicated. All the materials from this experiment were coded at the hatchery at the time of collection. Copies of this record are kept at the hatchery and at the regional offices.

The samples examined were from the following groups: 9, 10, 11, 12, 13, 14, 15, 16, 18, and 20 months. Atypical, bizarre and degenerative changes as well as hepatomatous pathology became generally more extensive in succeeding samples. The ninth-month livers showed only one group having hepatoma. From the twelfth month through the twentieth month, five of the seven groups were implicated. Hepatocellular carcinoma was found in at least one group from the fifteenth month through the twentieth month materials. No metastasis was observed grossly or microscopically in any of the materials. At the end of the year a total of 1260 livers was examined from the 9 month samples.

Liver tissues from the Hepatoma Survey conducted by the Branch of Fish Hatcheries were also processed and examined. These tissues were received from the following National Fish Hatcheries: Quilcene, Washington; Ennis, Montana; McNenny, South Dakota; and McNary, Arizona. No apparent hepatoma occurred in the livers of the ten 11- to 12-month old rainbow trout on each of the four commercial dry pellet diets. Summaries of the findings of both the Hagerman experiment and the Hepatoma Survey were submitted to the Central and Regional Offices.

"White spot" or "coagulated yolk" disease has been prevalent in hatcheries in the Northwest for many years. Numerous causative factors have been cited, i.e., water temperature, excessive pressure, rough handling, cations, etc. In order to have material available for morphological, histochemical, and histopathological studies, various methods were employed to induce this condition in the experimental laboratory. To date, however, results have been inconclusive.

Some diseased material was obtained from the Little White Salmon National Fish Hatchery and Coleman National Fish Hatchery. Detailed morphological study of these samples indicated that the outer membrane (vitelline) was void of white spots. The "coagulated" spots were located on the inner surface of the yolk membrane and/or interspersed throughout the yolk proper, appearing as snowflake-like flecks. A report is being prepared.

If the egg or yolk-sac fry had been processed without prolonged storage in alcohol, Bouin's fluid was found to be most satisfactory for histochemical and histopathological studies. A specific staining technique for the "white spot" is yet to be determined.

The toxic effect of formalin on rainbow trout has been known for some time. To determine the pathology of the toxicity, rainbow trout and silver salmon treated in 1:500 and 1:4,000 level of formalin were examined. The 1:500 level samples (both species) were taken at the following intervals: 10 minutes, 20 minutes, 30 minutes, and 1 hour. At the 1:4,000 level the rainbows were sampled at 1 hour, 1-1/2 hours, 2 hours, 2 hours plus 24 hours in fresh water, and 3 hours plus 24 hours in fresh water. The silvers were sampled at 1 hour, 1-1/2 hours, 2 hours, 3 hours, 5 hours, 3 hours plus 24 hours in fresh water and 5 hours plus 24 hours in fresh water. Definite pathology was seen on the lamellar (platelets) epithelium of the gills. Two changes were observed: one, hypertrophy and the other, separation of the epithelial layer from the lamellar supporting cells. Tissue changes of the gills of silvers at both levels were very slight; whereas, the epithelia of the gills of the rainbow exhibited extensive pathology in the 30 minute and 1 hour samples of the 1:500 groups and 2 hour and 3 hour groups at the 1:4,000 level.

Although there appear to be species specificity and millipore filterability differences, histopathological differences between the SRCD and OSD are still to be determined. Both diseases affect the kidney and pancreatic tissues. Additional experimental material will be available for further study.

As reported by the Virology section, IPN was experimentally substantiated for the

first time in the western United States, and for what appears to be the first time in the United States, IPN was also histologically diagnosed and confirmed by tissue culture inoculation from cutthroat approximately 18 months old. The typical extensive pancreatic necrosis and some striated muscle degeneration were observed in the fish from the State hatchery in Nevada.

PARASITOLOGY

Study of the biology and life history of the plerocerid snail Oxytrema silicula and its parasite consorts was a major activity throughout the year. This snail is a highly successful member of the biotic community in many Pacific slope streams in Washington, Oregon, and California. Considerable evidence has accumulated to suggest that the presence of large populations of Oxytrema with its attendant fish parasite risks, Nanophyetus salmincola and Sanguinicola spp., may mitigate against maximum productivity of co-resident salmonid fishes.

Although harmless to fish per se, Oxytrema, like many other snail species, is the first intermediate host of several trematode disease agents. Of immediate concern is Nanophyetus salmincola, which utilizes salmonid fishes as an intermediate host, and Sanguinicola spp. which invade and reproduce in the blood vascular system of fish hosts. Both agents arise as cercariae in the snails and, aside from their direct effects on fish hosts, may also act as potentiators of secondary microbial infections through their habit of direct physical penetration of fishes through body surfaces and pharyngeal elements.

Abernathy Creek, at Longview, Washington, site of the Salmon-Cultural Laboratory, was selected as a type study area to develop a basic understanding of the life history of Oxytrema and its inter-relationships with salmonids under natural conditions. Correlated studies were conducted at the Seattle laboratory to measure experimentally growth rates of Oxytrema and pathogenesis of Nanophyetus.

The Abernathy Creek system, some 29 stream miles, was explored throughout its reaches. Distribution and population density of Oxytrema was found to vary in relation to stream discharge and stream gradient. Chemical differences between tributaries were negligible with the exception of one tributary which was relatively iron rich; this tributary also supported a relatively high, dwarfed population of Oxytrema when compared with other tributaries and mainstream Abernathy. It was concluded, however, that optimal stream gradient, discharge and substrate were the factors which favored high survival, intense competition, and resultant dwarfness. It was found that other tributary streams in the Abernathy system do not support critically large snail populations and that mainstream, some 9 stream miles of the total system, supports in excess of 75 percent of the total standing crop of snails. Population estimates and migration rate tendencies indicate population densities in excess of one million snails per stream mile which tend to move upstream at a rate of 10 feet per day during the summer months.

Older age classes of snails present the major risk to potential fish hosts. It was demonstrated that the upper 20 percent of the size distribution of snails harbors 95 percent of the parasite risk; conversely, young-of-the-year snails (approximately 50 percent of the population) are not involved in transmission of disease. It is tentatively concluded that Oxytrema populations are manageable through chemical control and trapping. Experiments are projected for calendar 1963 to determine optimal control methods which will be consistent with the multiple uses of Abernathy Creek water.

Controlled experimental evaluation of Nanophyetus metacercariae in juvenile rainbow trout was effected. 1X, 2X, and 4X levels of infection were induced by maintaining known snail carriers in direct association with test fish. The 1X level of infection was represented by a snail to fish ratio which yielded an infection intensity in test fish approximately that observed in wild fish. Results showed that the 1X level of infection which prevails in wild fish of Abernathy Creek is non-pathogenic when measured in terms of its effects on mortality rate, growth response,

or stamina (stamina tunnel test, Salmon-Cultural Laboratory). In the case of 2X and 4X levels of infection, highly significant mortality rate differences were observed.

Evaluation of Clinostomum marginatum in lake-reared steelhead trout was essentially completed. The interaction of two year classes of trout and snails in Lynch Lake, Washington, was followed in collaboration with Washington Game Department biologists. It was concluded that complete eradication of fish and snails in this ecosystem was essential to prevent further spread of the disease to adjacent waters containing trout and Helisoma sp. populations. Rotenone poisoning for fish eradication was effected in September and followed up with copper sulfate poisoning in November to kill surviving fish and the standing snail population. Extremely valuable information was derived from this study with respect to the relative efficacy of rotenone versus copper sulfate as fish toxicants. The economics of this operation show clearly that copper sulfate, where its use is indicated, is superior to rotenone as a fish toxicant and significantly cheaper. Gill net sets after rotenone treatment were positive and fish activity was clearly evident. More than 100 trout were recovered in the immediate period following copper sulfate treatment; sustained gill net sets were negative. Periodic water sampling has been conducted since the copper sulfate treatment to determine the time to extinction of biologically significant levels of copper ion.

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

The Cortland laboratory is operated as a cooperative program with New York State and Cornell University. The research results are published annually by the State in a numbered series entitled "Fisheries Research Bulletin" These Cortland reports are more complete descriptions of the research results; they may be obtained from the Laboratory or the Department.

Cortland pelleted foods continue to rear trout economically and at low mortality rates. The fish are not of satisfactory quality.

The substitution of a portion of a base diet with inert cellulose flour increases diet efficiency in terms of calories and protein required for production. The replacement of the removed calories with corn oil increases the body fat. No evidence was found that dextrin, substituted for other calorie sources, is used by trout.

Vitamin K was found essential for brook trout.

The sensitivity of brook trout to a pyridoxine deficiency apparently decreases as the fish become larger.

It is believed that hatchery diets should duplicate the high phosphorus, low calcium content of fresh meat products for efficient mineral nutrition. Dissolved calcium is obtainable from hard waters but dietary calcium acts to bind phosphate in an unobtainable form.

Size grading of brown trout fails to increase the total weight gained over a 20-week period. These results duplicate those found earlier with brook trout.

Trouts fed meat diets have a higher content of serum protein than those fed meat-meal mixtures, and those held in colder water contained more serum protein than those held in warmer water.

Low water temperature significantly prolongs the clotting time of trout blood. There are species differences in blood clotting time.

EFFECT OF DIET COMPOSITION UPON GROWTH, SURVIVAL, AND QUALITY OF HATCHERY TROUT

Development of a complete dry food for trout

The purchase of a laboratory type food pelleting mill in the late fall of 1960 has facilitated the preparation and testing of controlled dry feed formulae as complete diets for trout. Started in July 1961, three of these pelleted diets have completed 18 months of testing.

The No. 1 pellet was a modified formulation of the Cortland diet that contained 43 percent protein and 1,250 calories per pound. The No. 2 pellet contained 37 percent protein and 1,105 calories per pound; the No. 3 pellet, 32 percent protein and 880 calories per pound. Each of the three pellets is being fed as a complete diet to brown trout under production conditions. The experiments started with 3,165 fish in each raceway. To prevent overcrowding, the weight and numbers of fish in the holding units are reduced at intervals. At this writing, each raceway contains approximately 250 fish. Each diet is tested in duplicate.

At the end of the 18 months experimental period, all diets have maintained the trout in fair condition. At times during the test period "blue slime" has been present and the fish have appeared listless. There have been no periods of excessive mortalities and the fish have handled reasonably well. Although we do not consider the fish to be in excellent condition the diets have produced them at good growth rates and with low losses.

There has been little difference in rate of weight increase: the fish fed No. 1 pellet have gained a total of 3,150 percent; those fed the No. 2 pellet, 2,880; and those the No. 3 pellet, 2,730 percent. These differences are not significant.

The conversion of food into fish flesh has been excellent. The total food conversion rate for the entire 18 months period is 1.76 for the fish fed the No. 1 pellet, 1.89 for those fed the No. 2 pellet, and 2.07 for those fed No. 3. Expressing the conversion in terms of calorie required to produce a pound of fish, places the No. 3 pellet as the most efficient, with a value of 1,817 calories per pound of fish produced; and No. 1 the least, requiring 2,206 calories. The No. 2 pellet is intermediate with a value of 2,089 calories. In terms of grams of protein required per pound of fish produced, the No. 3 pellet is most efficient, producing at a rate of 295 grams; whereas, No. 1 and No. 2 pellets produce at similar but less efficient rates of 325 and 343 grams of protein respectively for each pound of fish produced.

The average monthly mortality for the entire 18-month period was 0.45 percent for fish fed the No. 1 pellet and 0.36 for both the No. 2 and No. 3 pellet-fed fish.

Although these pelleted mixtures are not considered satisfactory in terms of fish health they have been successful and efficient in rearing trout when fed as complete foods. The experiments will be continued until the fish spawn, in an attempt to measure the affect of these foods on the viability of the reproductive products.

Utilization of calorie sources by trout for energy

Brook trout were fed three base diets containing 724, 598, and 529 calories per pound, and 27.3, 23.0, and 21.2 percent protein, which represented 67, 68, and 70 percent of the total calories as protein. The differing levels of calories and protein were obtained by a replacement of a portion of the first diet by inert cellulose flour. The calorie content of the last two diets was raised to the level of the first by the addition of corn oil and/or white dextrin. These additional calories reduced the percent of the total calories as protein to levels of 51 and 56 percent. At the end of 20 weeks the experiments were concluded and samples of trout preserved in formalin for protein, ash, and fat analysis to determine the purpose for which the dietary calories were used.

There was no difference in the growth rate or body chemistry of the trout fed the three base diets. The addition of corn oil to the two lower calorie diets increased trout "growth" rate (weight increase) significantly but the body analyses showed that at least a portion of the added food fat was deposited as body fat and therefore the increased weight of the fish may not be considered true growth.

The addition of white dextrin as a calorie source did not increase the weight gains of the fish in comparison to those of the trout fed the base diets and no changes in body chemistry were noted. It is concluded that the dextrin was not utilized as energy for true growth or for fat formation.

The two lower calorie diets produced trout substantially equal in weight to fish fed the higher calorie diet. In terms of efficiency the lower calorie diets were superior to the high calorie diet (2,272 and 1,851 vs. 2,688 calories per pound of fish produced). Similar results were found in comparing the protein required to produce a pound of fish. The diet containing the highest level of protein (27.3 percent) required 450 grams of protein per pound of fish produced; the intermediate diet (23.0 percent protein), 395; and the lowest protein diet (21.2 percent), 331 grams.

In these experiments employing similar base diets with differing calorie and protein content obtained by the substitution of inert cellulose flour, additional dietary calories as fat failed to produce increased true growth but did increase body weight by the deposition of body fat. In previous experiments in which the diet composition was altered by removal of individual ingredients this was not apparent, and added fat calories were utilized for energy for growth. Apparently at the levels of food fed, and with the ratio of ingredients used in the base diets, nutritional conditions were satisfactory, and the additional calories failed to increase the growth rate of the fish.

A repetition of these experiments with lake trout produced similar results. There was an increase in weight gained on oil-supplemented diets but the gain was found to be deposited fat and therefore not true growth.

THE VITAMIN REQUIREMENT OF TROUT

The vitamin K requirement of brook trout

The effects of deleting vitamin K from, or including sulfaguanidine in, Wolf's synthetic diet fed to brook trout was studied over a 16-week period. Sulfaguanidine was added to some of the diets as a means of controlling the possible role of bacteria in intestinal synthesis of vitamin K. The effect of these treatments was measured in terms of the clotting time and the hematocrit reading of the blood. By analyses of variance it was established that:

1. The deletion of vitamin K from Wolf's diet to which sulfaguanidine was added, significantly increased the coagulation time of the trout blood and decreased the hematocrit reading.

2. The deletion of vitamin K from Wolf's diet (which did not contain sulfaguanidine) significantly increased the blood coagulation time and decreased the hematocrit value.

3. The addition of sulfaguanidine to Wolf's diet significantly increased blood coagulation time and decreased the hematocrit values, independent of dietary vitamin K.

From these experiments it is concluded that vitamin K is an essential vitamin for brook trout, and sulfaguanidine has an effect similar to that of vitamin K withdrawal that possibly may be explained as resulting from control of bacterial flora of the tract.

Interestingly, a reduced growth rate during the last 4 weeks of the experiment was exhibited by those fish fed diets lacking dietary sulfaguanidine. During this period there was an outbreak of bacterial gill disease in the hatchery building. Although the disease was not confirmed in these experimental fish, it is possible that there was a differential effect on growth of a disease that we were not aware of at the time.

The pyridoxine requirement of brook trout

Past experiments indicated that the resistance of brook trout to a pyridoxine deficiency increased as the fish became older. This increased resistance might be correlated with increased fish size, fish age, or with seasonal changes associated with metabolic cycles. Experiments still in progress suggest that the increased resistance may be correctly correlated with increased fish size.

Three series of pyridoxine deficiency experiments have been undertaken with fish of about the same age. In each of the latter series of experiments the fish used were approximately twice the size of those in the previous series. All of these fish were fed diets lacking B6 until 50 percent died. For fish 3.64 grams in weight, 8 weeks were required; for fish 6.11 grams, 12 weeks; and for fish 11.6 grams, in excess of 12 weeks (still in progress).

In the last and current series, it was possible to run, simultaneously, an additional trial using trout that averaged 19.9 grams in weight. These larger trout appear less sensitive than the fish averaging 11.6 grams in weight. It is on the basis of this evidence that we presently favor a correlation with fish size as the explanation for differences in observed sensitivity to pyridoxine deficiencies.

MINERAL METABOLISM OF TROUT

Acclimation of trout to changes in the mineral content of surrounding waters

A prior experiment indicated the inability of brook trout to acclimate to an abruptly lowered calcium content of their water environment. Since calcium appears to be an indispensible ion in aquatic environments, a compilation has been made of data from mineral experiments conducted at Cortland during the past several years in which acclimation was not a specified variable although it was implicit in the various experimental designs. These data corroborate the results of the prior acclimation study, and support the following observations:

1. Bone mineral appears to serve as a reservoir that assists in the relative ionic independence of trout in hypotonic fresh water. In the presence of dissolved calcium, the trout can practice discriminatory regulation of each

ion (e.g. chloride, cobalt, sulphate) separately, and thereby remain independent of the external environment while maintaining, presumably through hormonal control, the necessary constant internal environment.

2. Brook trout acclimated to concentrations of calcium sufficient to maintain normal behavior will suffer distress and mortalities when transferred abruptly to water of calcium content below three to five parts per million. The observed symptoms of the distressed fish indicate a condition much like the increased excitability and convulsions of tetany in higher animals caused by a low internal calcium concentration bathing the cell walls.

3. The stress symptoms of trout are aggravated by increased water temperature. These effects are pronounced at water temperatures that are within the acclimation range of fish and that are not usually lethal. This stress is felt to be attributable to the increased metabolic requirements (and cellular activity) of trout in warmer water. A drastically lowered mineral content of the water also tends to cause an increased metabolic rate. A combination of starvation, high water temperature, and low mineral water can be lethal.

4. Reduction of water temperature to near the freezing point reduces the calcium requirement and permits brook trout to acclimate, at least temporarily, to lowered mineral concentration. Since the utilization of food minerals remains largely independent of the lowered water temperature, the dietary minerals available to a fish with a lowered metabolic rate could assist in maintaining the storehouse of minerals in the bone. At a high water temperature of 66° F., a depletion of this storehouse can be measured within 48 hours after transfer of trout to a very soft (0.8 ppm calcium) water.

Comparative absorptions of dietary minerals by trout

A review of previously reported experiments, based on short-term forced feeding of selected anions and cations permits the following generalizations:

Brook trout are capable of extensive gastric absorption of the minerals from these sources. The presence of excess dietary fat, or of minerals that form insoluble salts, limits the trout's gastric absorption and, thus, the eventual utilization of minerals. Additional calories provided by extra glucose in the food increases the gastric absorption of minerals.

Practical hatchery diets probably should duplicate the high phosphorus, low calcium content of fresh meat products for efficient mineral nutrition. Dissolved calcium is directly obtainable from reasonably hard waters; dietary calcium acts to bind the phosphate in an unobtainable form.

Chloride in the diet is nearly completely exchanged with the chloride of the body fluids, while sulphate is retained to a limited extent by structural tissues. Trout may be unable to use dietary cobalt in the inorganic form since an almost complete turnover and excretion of labeled cobalt was measured within 48 hours after feeding. Apparently only trace quantities of cobalt and sulphate need appear in the trout diet; both of these minerals are readily obtainable from water.

Other minerals are rapidly excreted under conditions of increased metabolism caused by high water temperature or low calcium water. Both the turnover and utilization of the highly available minerals are promptly influenced by changes in the metabolic activity of the fish, although the final tissue deposition from one meal of labeled phosphate was independent of water temperature from 35° to 66° F. Cold water favored a longer retention of the phosphate within the trout stomach.

During these short term experiments, no change in quantity of any mineral was detectable by ordinary chemical analyses, probably because of the small daily growth of the fingerling and yearling fish. Rapidly growing trout embryos do show changes in total mineral content as the mineral sources in the water are altered. For slower growing fish, these experiments measured an exchange of dietary calcium, phosphorous, and sulphate with that of the skeletal and muscular tissues, and an

exchange of dietary chloride with that of the extracellular fluids. The appearance and concentration of all minerals in the blood of trout provided a convenient measure of these exchange processes.

EFFECT OF PHYSICAL FACTORS ON THE GROWTH OF HATCHERY TROUT

Effect of grading on the weight gained and survival of brown trout

This laboratory previously reported experiments upon brook trout showing that differences in the rates of weight gain of several sizes of fish were not dependent upon grading. In recent experiments, four lots of brown trout were graded into "small" and "large" trout and held for 10 weeks after which they were re-graded into "small", "medium", and "large" trout and held for an additional 10 weeks. Four other populations of brown trout were held ungraded as controls. The experimental methods were essentially like those previously reported for brook trout.

No increased weight gains resulted from grading these brown trout. The combined gains of segregated size groups were essentially similar to the gains of ungraded control lots. The rates of conversion of food into fish flesh were similar (averaging 2.8 for all groups) and were excellent for the meat-meal diet fed. As was concluded for brook trout, there is no valid reason for grading brown trout to increase the total weight of fish produced or the efficiency of the foods fed. The value of grading fish to meet management requirements or to improve certain hatchery operations is not questioned.

Hand counting of the trout at the start, during, and at the end of the experimental period failed to show cannibalism in either the graded or ungraded populations. It was feared that cannibalism might be a factor in studies with brown trout.

The amounts of food fed to each of the experimental groups varied with the average fish size, and were obtained from standard feeding tables basing food allowances on fish size and water temperature. The amount of food fed to the ungraded controls almost exactly equaled that fed to the graded fish. The low conversion rates of food into fish flesh confirm the validity of the food levels selected and thus the validity of the trout feeding tables in common usage. Similar results were reported in the previous experiments with brook trout.

CHEMICAL COMPOSITION OF TROUT BLOOD

Changes in blood coagulation time and hematocrits caused by water temperature, species, and diet

The effects of diet (pellets vs. meat-meal mixtures), species (brook, brown, and rainbow trout), and water temperature changes (Avg. 52° F. vs. 37.3° F.) upon the coagulation time, hematocrit, and prothrombin time of the blood were measured.

Effect on coagulation time:--Differences between species were highly significant, brown trout exhibiting the longest coagulation time and rainbow trout the shortest. Compared to the blood coagulation time of the fish sampled directly from the higher water temperature there was a mean increase of 52.2 percent in coagulation time exhibited by fish transferred to and held in the colder water for a minimum overnight time of 15 hours.

No significant differences were caused by diet, however, a highly significant interaction was found between diets and water temperature. While a mean 74.9 percent increase in coagulation time occurred in the blood of pellet-fed fish when they were transferred from the higher to the lower water temperature, a mean of only 29.4 percent increase occurred when the fish fed the meat-meal diet were transferred from the warmer to the colder water. No explanation is offered for these differences.

Effect on microhematocrit values:--Differences in microhematocrit percentages at the two water temperatures were highly significant. A mean decrease of 10.9 percent was exhibited by fish in the colder water. A significant interaction was found between diet and water temperature. Whereas a mean 22.2 percent decrease in microhematocrit was found in the

blood of pellet-fed fish after transference to the colder water overnight, an increase of 1.6 percent was found for the blood of the fish fed the meat-meal diet after being transferred to the colder water.

Prothrombin time:--The plasma-thromplastin-$CaCl_2$ mixtures always coagulated within less than 1.5 minutes in plasma obtained from all three trout species held in the higher water temperature and fed either diet. Samples from trout held overnight at the lower water temperature, however, failed to coagulate consistently even when stirred for as long as 18 minutes. The introduction of an additional 0.1 m. of $CaCl_2$ at the end of four or more minutes following the initial addition of $CaCl_2$ to the plasma usually caused coagulation to occur in one minute.

Effect of water temperature, diet, and activity on the daily fluctuation of total blood protein

Total serum proteins were measured in brook trout fingerlings using a three factorial design in the study with two diets, (meat vs. meat-meal mixture), two water temperatures (constant 47° F. vs. Avg. 51° F.) and four time periods, (2-hour intervals over an 8-hour period).

Four hundred microliters of blood were removed by cardiac puncture from each fish using a laboratory made glass pipette. The serum from three fish was pooled to make one serum sample for analyses. Two pooled samples were taken from each group of fish during each sampling period and duplicate protein determinations made upon each pooled sample. The pooled samples of serum were analyzed in a Spinco Ultramicro Analytical System, requiring 10 microliters of serum for each analyses.

Analyses of variance established highly significant differences due to the effects of diet and water temperature. Blood serum from fish fed the all meat diet contained more total protein than that from fish fed the meat-meal mixture and fish held in the colder water had a somewhat higher total serum protein than those held in the warmer water. Time as such had no effect on the protein level of the serum.

S T A F F

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Cook, Washington
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Emphasis was continued on determination of basic nutritional requirements of salmonids and respective role of each in metabolism.

Impaired lipid metabolism was confirmed as the most logical primary or secondary precursor for hepatomagenesis.

After 20 months of feeding, cancer appeared in fish fed fat extracted from commercial ration, from the neutral lipid fraction of that feed, and when 11 individual chemical compounds were fed to young rainbow trout. Preliminary evidence indicated that nutritional treatment plus hormones would eradicate liver tumors from tumor-bearing rainbow trout.

The Vitamin E requirement of chinook salmon was confirmed as slightly below the tentative value previously indicated.

Use of aminogram techniques with chinook salmon and rainbow trout indicated reliable correlation between indispensable amino acids and the free amino acids present in blood serum.

The minimum protein requirement for maintenance of salmon and trout was determined and protein quality measurements were tested for a number of dietary ingredients.

Essential fatty acids for chinook salmon were tentatively established with the minimum linoleic and linolinic acids as well as total fat requirements for chinook salmon recorded.

Digestive enzymes were further characterized. Alkaline phosphatase activity and characteristics were determined and used to complement mineral feeding studies.

Inorganic requirement studies with salmon were emphasized in experimental feeding program this year with major emphasis on calcium, phosphorous and zinc interrelationships.

Work on water as a dietary essential was extended and the general concept confirmed.

Water chemistry as a factor in white spot disease in chinook salmon fry was continued with results on high zinc or copper directly affecting the rate of white spot appearance.

Thyroid function in chinook salmon and rainbow trout was investigated and radiodine uptake measured in thyroidectomized and normal fish.

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

The histopathology of chemically induced or sublimital pharmacologically induced hepatoma was continued and further characterization of microanatomy of different types of observed neoplasms recorded.

The library of histopathological material was expanded with approximately 5,000 new slides of specific descriptive tissues.

Cooperative research ventures between laboratories interested in donating their talent and facilities for solution of particular problems in intermediary metabolism were organized and programs initiated covering intermediates in AAF detoxification; dimethylnitrosamine intermediates; hemoglobin characterization; argiylarginine as a tissue growth promoter; Aflatoxin effects in fish; amino acid requirements of channel catfish; polycyclic lipids in carcinogenesis; carbonyls in fish metabolism; erythrocyte transketolase and transaminase for vitamin status; antigen reagents for racial identification; and aminogram projection of indispensable amino acid patterns.

Two new laboratory areas were completed, one for physiology and the other for biological assay experiments.

Alkaline phosphatase of salmon blood

Determination of alkaline phosphatase activities in the blood sera of adult salmon was continued in 1962 using the p-nitrophenylphosphate assay method and refinements in technique to reduce experimental error. Results confirmed the preliminary report of 1961 of species differences and changes during the spawning migration. The pertinent data for the different groups are given in table 1. The range of value in each group shows a big variation among individuals. However, calculations of the t values revealed at the 1 percent probability level the following significant differences between groups:

(1) sea-run silvers had higher alkaline phosphatase value than the sea-run chinooks.

(2) sea-run chinooks had higher values than the spawning chinooks.

(3) spawning chinook males had lower values than spawning chinook females.

It is questionable whether there was a significant decrease in the values for the spawning chinook females as compared to the sea-run chinook females, so the main change during the spawning run was the decrease in values for the males. There was no significant difference in values for the sexes of the sea-run groups. There was no correlation between the phsophatase level and the size of fish or the degree of hemolysis, so these factors probably do not account for the big variation among individuals.

Chromatography of salmon caecal proteases

The chromatographic technique used for the separation of the salmon digestive enzymes has been modified to incorporate continuous gradient salt elution and has been extended to include chromatography of the cationic components (Fraction 1) on CM-cellulose. Thus is obtained a complete picture of the main TAME- and ATEE-hydrolyzing activities free from possible artifacts of stepwise elution. The chromatogram of the activities absorbed on, and then differentially eluted from, DEAE-cellulose is given in figure 1. The fractions correspond to and support the validity of the previously reported Fractions II, III, and IV and the numbering system is retained. The improved technique gives the additional information of an indicated heterogeneity of Fraction IV to include two rather diffuse sub-fractions a and b. Supporting evidence that Fraction IV is peculiar to fish was obtained in the chromatography of extracts from rat intestine which showed no detectable Fraction IV activity.

A chromatogram of the Fraction I activities chromatographed on CM-cellulose, after recovery from a DEAE-cellulose breakthrough volume, is shown in figure 2, and consists of two ATEE-hydrolyzing and one TAME-hydrolyzing activity. A corresponding chromatogram of bovine trypsin and alpha-chymotrypsin in figure 2, shows their similarity to two of the fish enzymes.

In addition to the above endopeptidases, a considerable amount of carboxypeptidase A activity (hydrolyzes carbobenzoxy glycyl-L-phenylalanine) was present in the extracts. The problem of its true position in the chromatograms has not been completely resolved because of analytical inconsistencies and an apparent flooding of the DEAE-cellulose columns with the activity in extracts containing levels of endopeptidases desirable for chromatography such as used for figure 1. It seems that a major portion of the activity was coincident to or closely associated with components II and III. When more dilute extracts were used to avoid flooding about one-fifth of the carboxypeptidase activity still was not absorbed on DEAE-cellulose and was carried through to the CM-cellulose chromatogram in the 1c region.

Effect of certain metal ions on the embryonic development of chinook salmon

An experiment in which the effect of zinc and calcium ions on embryonic development was described in the annual report for 1961. Experimental results since this report may be summarized as follows:

Table 1:--Blood alkaline phosphatase of groups of salmon

Identity of Group	Number of Individuals	Range of Values*	Mean Activity*	Standard Deviation
Sea-run Silvers	49	44-322	114.1	50.9
Males	31	44-322	113.8	55.7
Females	18	50-225	114.6	41.4
Sea-run Chinooks	59	22-131	58.1	16.4
Males	40	22-88	57.3	14.5
Females	19	31-131	59.6	19.6
Spawning Chinooks	50	17-100	42.9	13.9
Males	26	17-50	35.9	8.3
Females	24	34-100	50.5	14.9

* Expressed as change / minute in absorbance under assay conditions X 10^4.

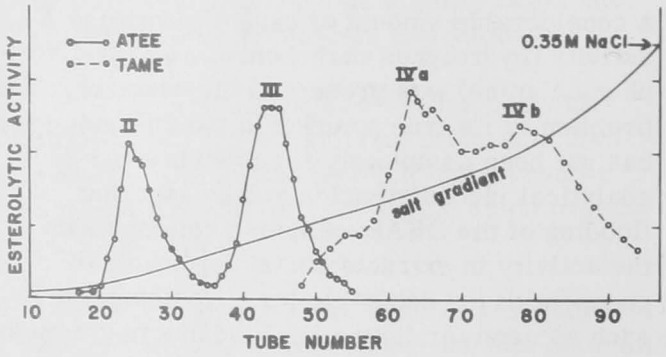

Figure 1:--Chromatogram of anionic esterolytic activities of salmon caeca.

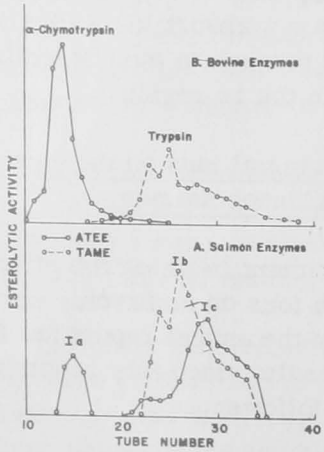

Figure 2:--Chromatograms of cationic esterolytic activities of salmon caeca and of bovine enzymes.

(1) The incidence of "white spot", a yolk abnormality, was directly proportional to the level of zinc present in the water; calcium having no effect;

(2) No significant fry mortalities occurred, indicating that if zinc is a vector in this disease then either (a) a greater amount is necessary to cause an intense enough reaction to result in death or (b) an additional factor such as copper ion which is reported to act synergistically with zinc, must be present or (c) the maximum effect occurs prior to "eyeing";

(3) Zinc added to the water was retained in part by the fry; the addition of calcium ion reversed this trend;

(4) Zinc ion both delayed and prolonged the rate of hatch, an effect which was canceled by calcium ion.

In the current egg cycle a far more extensive experiment is being conducted in order to answer some of the questions raised in the work of previous years. The incubation setup is similar, i.e. plastic bottle incubators are being used; metal ion concentrations are maintained by metering a concentrate directly into the water line supplying the incubators; an improved means of controlling water flow was attained by using steel needle valves. The incubation design is illustrated schematically in figure 3. Duplicate bottles containing approximately 1,000 green eggs were incubated under the influence of various metal ion concentrations as shown at the top of the following page.

An additional group of the same lot of eggs was incubated by the usual procedure in the hatchery troughs. When all groups were eyed the dead eggs were counted and the eggs from one of the duplicate bottles from each group were transferred to hatching trays in troughs and a new group was started from the eggs which had eyed in the troughs. Dead eggs were removed weekly from all groups until hatching commenced. Hatching rate was followed by removing the hatched fry to a

Group No.	1	2	3	4	5	6	7	8	9	10	11	12
Zinc added ppm		.05	.10	.20			.05	.10		.05	.10	.20
Cu added ppm					.01	.02	.01	.01		.01	.01	.01
Ca added ppm									20	20	20	20

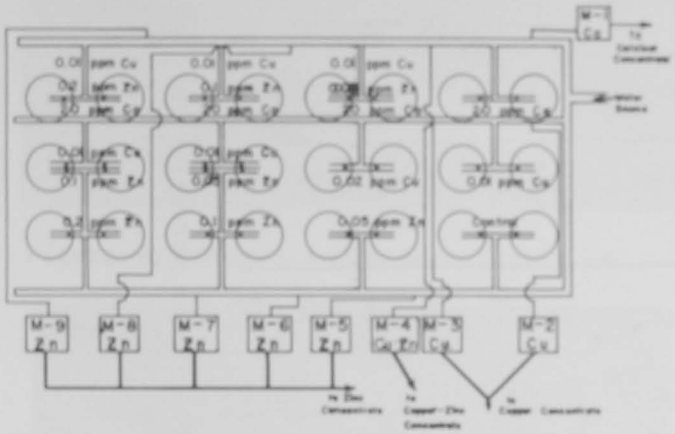

Figure 3:--Incubation Design

covered beaker at the bottle overflow twice daily during the hatching period. When hatching was nearly complete the fry were returned to the incubator and unhatched eggs placed in the beaker. Mortality rate of hatched fry was followed by removing dead fry at weekly intervals or in those instances where mortality rate was excessive, at daily intervals.

Table 2 shows the hatching rate and mortality rate of the subgroups which were under continuous treatment from the green egg stage. The prolonged hatching period of the groups receiving zinc is again noticeable. An unexpected finding was the acceleration of hatch caused by copper ion. As before, the addition of calcium overcame the prolonged and delayed hatch caused by zinc. The extreme sensitivity of both eggs and fry for copper ion is apparent as is the synergistic effect of copper and zinc--compare accumulated mortality of 2 and 5 with 7, or 3 and 5 with 8. The antagonistic effect of calcium ion for heavy metal ion toxicity initially appeared to be confirmed--compare mortality in group 10 or 11 with 7 or 8 until December 10. The rapid increase in mortality in groups 10, 11, and 12 was a dramatic occurrence and only after checking and rechecking the metering rate and ionic concentrations was it concluded that the effect was real and not artifact.

Zinc or copper caused a number of significant changes in embryonic development other than the obvious ones shown in the table. Either or both caused an apparent hardening of the egg shell which prolonged the hatch, i.e. a small opening would be formed in the egg shell and the larvae would be able to get its head or tail or part of the yolk sac out and there it would stay several hours before hatching was complete --in many instances death occurred at this stage. This hardening could be due either to the formation of a protein complex with the heavy metal ions which resist attack by the hatching enzyme or the copper or zinc may poison the enzyme itself thus reducing its effectiveness in hydrolyzing the normally tough keratin material of the egg shell. Also most noticeable was the difference in shape and firmness and color of the yolk of the newly hatched fry. In normal fry the yolk is quite oblong, firm and bright orange. In groups other than 1 and 9 there was a significant number of fry having round, pale, flabby yolks. The yolk lacked sufficient firmness to hold shape if the fry was not supported by water. The incidence of this condition, while not specifically measured, was significant in groups 2, 3, and 4 and predominant in the remaining groups except for group 9. Frequently white spot was associated with the condition. The condition is illustrated in figure 4. There is a pronounced difference in rate of development following the hatch. As of this date, December 19, 1962, groups 1, 2, 3, 4, and 9 are essentially healed and ready to go into troughs while the remaining groups show little development in the side wall and the yolk is scarcely absorbed. This is illustrated in figure 5. These remarks, regarding stage of development, refer to the groups which are in the bottle incubators. The groups which were transferred to trays after eyeing under treatment did not show nearly as much difference nor has there been a comparable fry mortality rate. The fry mortality rate of the subgroups on which treatment started after being eyed is nearly equal to the subgroups which were under treatment for the entire incubative period.

Table 2:--Effect of metal ions on rate of hatch and mortality of chinook salmon eggs and fry

Date 1962	Heat Units	Group 1		Group 2		Group 3		Group 4		Group 5		Group 6		Group 7		Group 8		Group 9		Group 10		Group 11		Group 12	
		H	M	H	M	H	M	H	M	H	M	H	M	H	M	H	M	H	M	H	M	H	M	H	M
10-29	519		2		4		3		3		4		55		10		30		5		6		2		2
11-6	664		3		6		6		5		6		76		15		41		7		10		3		3
11-13	790		3		7		7		6		8		92		21		46		7		12		7		3
11-19	893		4		10	1	9	1	8	25	10	.4	98	23	32	18	57	1	9	16	13	18	8	47	3
11-20	911	8		4		19		5		74		.7		46		28		5		75		69		64	
11-21	935	93		64		43		15		87		.9		59		37		79		86		89		95	
11-22	950	94		80		54				87				61		39		90		86				95	
11-23	964			82		71		68		89				64		42								96	
11-24	987			84		86		89						64		42									
11-25	1005			84		90		91																	
11-26	1018		5		19	91	12		10		15	1.6	99		47		66		15		17		15		17
12-3	1144		6		22		12		11		25				72		73		17		18		16		17
12-10	1268		6		22		12		11		36				90		84		17		42		27		48
12-17	1394		7		22		12		12		55				98		96		17		85		81		83
12-19	1430		9		23		12		12		56				99		98		20		91		87		94

Numbers indicate percent of starting egg population hatched (H) or mortality (M)
See text for treatments accorded each group

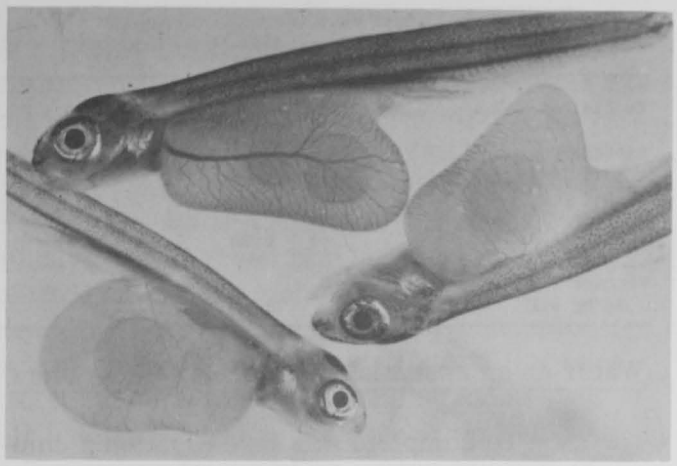

Figure 4:--Normal and abnormal yolk sac.

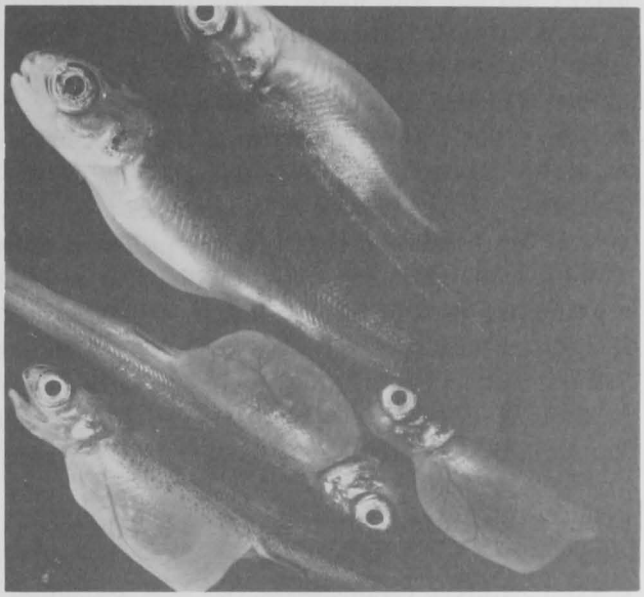

Figure 5:--Comparative rate of development.
Top two fish, Control
Bottom three fish, Group 7a

Iodide requirements of chinook salmon

The iodide requirement of chinook salmon fry was determined. Five replicate lots of fry were fed diets containing varied levels of iodide for a 24-week period. At the termination of the feeding trial the amount of stored iodine was determined in 10 randomly selected fish from each lot. The thyroid region from an additional sample was sectioned and stained for histological examination to determine the occurrence and extent of hyperplasia. Pertinent data are summarized in table 3. It can be seen that iodide had little effect on growth, food efficiency or mortality but that much less iodide was stored from a diet containing .01 mg percent iodide than from all higher levels. The results indicate that the iodide requirement for chinook salmon lies between .01 and .06 mg per 100 gms dry diet.

Table 3:--Iodide requirements of chinook salmon

	1	2	3	4	5
Iodide level in diet Mg percent	.01	.06	.11	.61	1.01
Average gain grams	7.57	7.97	7.96	7.81	7.95
Food efficiency	1.02	1.03	1.04	1.01	1.04
Percent mortality	4.6	4.7	3.5	4.1	3.3
Iodine stored µg/kg fish	37	96	105	111	114

Fat requirements of chinook salmon

The comparative growth potential of three types of fat--vegetable, mammal and fish--was investigated using chinook salmon fry as the test animal. Replicate lots of chinook salmon fry were fed diets containing 2.5, 5.0, 10.0, and 15.0 gms of each fat per 100 gms of dry ingredients for 16 weeks. All diets were held isocaloric at 360 calories per 100 gms dry ingredients. A control lot was fed a fat free ration at the same caloric density and all were compared with the complete test diet standard for this station. Although the statistical analyses have not been completed it appears that:

(1) The growth response to the fat free ration is less than any of the diets containing fat, confirming again that fat is essential for other than energy requirements;

(2) The growth response of herring oil is greater than corn oil or lard;

(3) Growth of fish on 5 percent and 10 percent herring oil diets is greater than on 2.5 percent herring oil; and

(4) Fifteen percent herring oil shows some growth inhibition. Performance of the fish on the various diets is presented in table 4.

Table 4:--Comparative growth response to corn oil, lard, and herring oil by chinook salmon fry.

Percent oil in diet	2.5	5.0	10.0	15.0
CORN OIL				
Average gain	2.01	2.18	1.97	1.79
Food efficiency	.46	.48	.59	.57
Performance index	.91	1.06	1.16	1.01
LARD				
Average gain	1.74	1.81	1.97	1.86
Food efficiency	.49	.54	.53	.59
Performance index	.86	.97	1.04	1.10
HERRING OIL				
Average gain	2.50	2.99	3.11	2.07
Food efficiency	.45	.52	.72	.61
Performance index	1.12	1.55	2.24	1.26

CONTROLS	Fat Free Diet	Complete Test Diet
Average gain	1.40	2.82
Food efficiency	.37	.62
Performance index	.52	1.74

Vitamin E requirements of chinook salmon

In brief summary, previous investigation had shown that Vitamin E was required for growth and health of chinook salmon fry and that the requirement in a test diet containing 5 percent herring oil triglycerides as a fat source was probably less than 10 mg Vitamin E per 100 gms dry diet. The current studies were designed to confirm previous findings and to extend the range investigated to lower levels. Replicate lots of chinook salmon fry were fed diets containing 2.5, 5.0, 10.0, 20.0, and 40.0 mg alpha tocopheral per 100 gms dry ingredients for 24 weeks. At the termination samples were removed for: (A) Carcass analysis; (B) Vitamin E storage; and (C) Histological examination. No significant difference was noted in weight gain between the groups nor in proximate analysis of the terminal samples. There was a marked difference in Vitamin E storage. Histological examination has not been completed. A growth summary of the two years studies is presented in table 5.

Table 5:--Growth response and storage of Vitamin E and chinook salmon fry.

Mg % E in diet	0	2.5	5.0	10.0	20.0	40.0	80.0
Average gain 1961	3.83			6.03	6.49	6.54	6.09
Average gain 1962		7.21	7.49	6.98	7.31	7.47	
Storage mg/kg oil		23	24	78	121	248	

Water as an essential in chinook salmon diets

To complete this investigation a comparison of the rate of moisture uptake into the stomach of chinook salmon, rainbow trout and silver salmon was made. The rate of passage of food through the stomach was also determined. It was shown that both the rate of moisture uptake and the rate of food passage was greater for the trout and silver salmon than in chinook salmon. These observations could be an explanation for the observed difference in response of these species to dry pelleted diets. They also support the hypothesis advanced that a principal reason for the failure of chinook salmon to adequately respond to dry diets lies in their inability to either ingest or otherwise moisten the dry food consumed rapidly enough for digestion to proceed at a satisfactory rate.

Effect of chemical differences in hatchery water supplies on stream survival of hatchery reared rainbow trout

The current investigation is a continuation of a broad project dealing with the factors which influence the stream survival of hatchery reared rainbow trout in which this laboratory is cooperating with the California-Nevada Sport Fishery Investigations. The objectives of the current investigation are: to determine if the concentration of the major electrolytes in the body of rainbow trout are influenced by their concentration in environmental water supplies; if a concommittant change in electrolyte concentration occurs following a change in water chemistry following stream planting or transfer between hatcheries; if changes during food deprivation or stream residence can be correlated to stream survival. Determinations conducted included: hematocrit, proximate analysis, inorganic ions in (a) total carcass

(b) muscle (c) serum. Sampling times were (or will be): (1) at the hatchery, following 3 days of food deprivation, (2) on arrival at Convict Creek,(3) in stream pen (virtual food deprivation) after 1/12, 1/6, 1/4, 1/2, 1, 3, 7, 14, 28, 42, 84, and 170 days,(4) from the stream sections at 42, 56, 70, 84, 170, and 340 days. Fish from four different hatcheries selected on the basis of extremes of water chemistry are included in the above sampling; each sample consisted of five fish. Fish were also sampled at five additional hatcheries and at four hatcheries which had exchanged fish 3 days previous to sampling. Also samples of stream resident rainbows were obtained from Convict Creek and another stream adjacent to the area.

Analyses completed to date include: serum electrolytes on all sera collected (approximately 500 samples) through the 170 day sampling time, (table 6); muscle electrolytes except for chloride of the hatchery collection and the first seven sampling times at Convict Creek; the remaining muscle samples have been ashed and prepared for analyses. A statistical treatment and evaluation of most of the data has not as yet been attempted but a few of the observations may be mentioned.

A. Hatchery Samples: (1) In those groups where food deprivation for 3 days prior to sampling was not complete, serum potassium values (but not calcium and sodium) were higher. (2) There appears to be a correlation between serum sodium levels and sodium concentration in the environmental water. This is not true of calcium and potassium. (3) Transferring fish from low to high sodium water and vice versa resulted in a significant and like change in serum sodium.

B. Fish transferred to Convict Creek: (1) Serum electrolyte levels in the samples taken from the stream were generally higher than like samples from the pen held groups probably indicating the influence of feed. (2) In the pen held groups two of the hatchery stocks showed a significant decrease in serum sodium; in one there was no significant change and in the fourth group the change was so dramatic that it deserves separate consideration. (3) In this group there was a decrease in all serum cations measured (but not chloride) which fell to half of their initial values within 24 hours following their placement in the stream pen. Understandably, these fish were extremely distressed and suffered a rather high mortality rate. In searching for a cause of this abnormal behavior it was found that the group was severely infested with the blood parasite Sanguinicola davisii. Eggs of this parasite had completely clogged the gill capillaries. Two theories are advanced for the resultant electrolyte losses. First that the plugging of the capillaries resulted in a lack of ingress for metal ions, an effect which, when coupled with a probable requirement for these ions by the developing parasite eggs would yield a rapid net loss to the host. The second possibility is that the developed miracidia which breaks through the gill membrane to leave the host fish would in effect leave a hole in the osmotic barrier permitting the direct loss of electrolytes. The latter does not seem to be implicated in this instance since there was not a concommittant loss in either chloride or blood values. It is known, however, that mortalities result due to hemorrhaging at the gills in heavily infested fish. Some electrolyte changes may be seen in table 6. This project will continue until May 1963.

Endocrine regulation of electrolytes in rainbow trout

Two sets of experiments were completed. In one, aldactone, a specific aldosterone inhibitor was injected into test fish and the levels of serum and muscle electrolytes compared with controls. In the second group metapirone, which selectively inhibits the 11-hydroxy adrenal steroids in mammals, was force fed to mature rainbow trout at the rate of 70 mg per kg body weight per day for 3 months. At the termination of the experiment muscle and serum electrolyte levels were compared with controls. In both experiments endocrine glands were removed and fixed for future histological examination. It was found that aldactone injection resulted in a retention of sodium and a pronounced darkening of the fish relative to the controls. The change in pigmentation is illustrated in figure 6. In the metapirone

Table 6:--Serum electrolyte changes in rainbow trout from four hatcheries prior to and during residence in Convict Creek.

PEN

	Mocassin Creek			Moorehouse Springs			Hot Creek			Darrah Springs		
	Na	Ca	K	Na	Ca	K	Na	Ca	K	Na	Ca	K
Days												
H*	150	5.6	1.87	160	5.5	1.97	157	5.8	2.38	153	5.5	2.04
0	129	4.3	.82	151	6.7	1.20	168	5.6	1.06	159	5.3	1.58
1/12	115	3.9	.59	143	7.0	.77	169	6.1	1.11	160	5.9	1.23
1/6	143	5.9	.71	131	7.1	1.20	158	5.6	1.31	97	3.3	.71
1/4	135	4.6	2.41	143	6.2	2.65	161	5.8	.84	88	3.4	1.38
1/2	124	5.1	1.36	148	6.3	1.44	156	6.2	.97	86	2.8	.64
1	103	5.0	2.92	140	6.0	3.88	158	6.5	2.33	77	2.7	0.0
3	123	4.3	.56	145	6.9	1.91	154	6.1	1.09	98	3.7	1.55
7	127	4.7	.77	146	5.6	1.62	150	5.5	1.26	119	4.4	1.12
14	137	4.6	1.16	150	6.0	.90	163	6.2	.77	98	4.2	1.02
28	146	4.3	4.11	166	5.9	1.43	171	5.5	1.90	158	5.5	.82
42	150	4.6	.91	153	5.3	.87	158	5.3	.82	159	4.8	1.19
84	132	3.0	.48	151	4.7	.94	158	5.4	1.24	147	3.8	.72

STREAM

	Mocassin Creek			Moorehouse Springs			Hot Creek			Darrah Springs		
	Na	Ca	K	Na	Ca	K	Na	Ca	K	Na	Ca	K
Days												
28	166	6.1	1.78	146	6.1	1.97	153	6.0	1.63	166	6.0	1.32
42	148	5.5	1.52	147	5.7	1.56	164	5.8	1.58	168	6.6	1.30
63	144	5.3	1.39	145	5.6	.96	160	6.2	1.91	171	4.8	1.62
77	160	4.5	1.00	158	6.0	5.55	154	4.6	1.75	176	5.4	2.84
84	158	4.2	.51	148	4.6	.71	156	6.0	.73	159	5.4	.51

*Hatchery Sample
Each number represents average of 5 individual samples.
Numbers represent millequivalents of ion per liter of serum.

experiment no significant electrolyte changes were observed. There was a difference in muscle magnesium stores but the difference seemed to be due to high levels in the control fish rather than a loss of the element in the treated fish. Histological evaluation of the endocrine glands in these experiments has not been completed.

Fat sources for salmon

Corn oil, peanut oil, and safflower oil were compared as fat sources in our complete test diet. The basal ration, containing 30 parts casein, 12 parts gelatin, was common to all three diets. The diets differing only in fat source, were fed ad libitum to silver salmon for a period of 10 weeks. Results are summarized in table 7.

The trial was terminated after 10 weeks due to the presence of kidney disease in several troughs. The apparent minor superiority of peanut oil may be explained by a lower incidence of kidney disease. The results do not warrant changing the source of fat in our complete test diet. Safflower oil seemed to be suitable as a source of fat over the course of this trial.

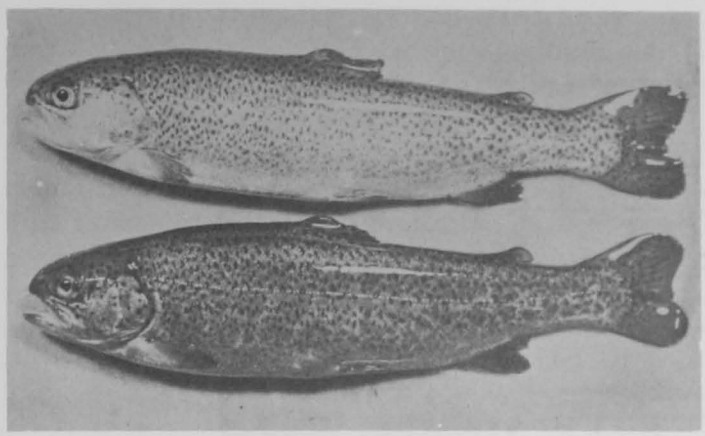

Figure 6:--Pigmentation change in aldactone injected rainbow trout (bottom) versus control (top)

Table 7:--Comparison of vegetable fats in silver salmon diets

Fat	Corn Oil	Safflower Oil	Peanut Oil
Total Wt. gain	336.7	322.8	344.9
Average gain	4.031	4.371	4.434
Food Fed	625.7	656.3	666.1
Food efficiency*	0.538	0.492	0.517
Performance index**	2.17	2.15	2.29
RBC count	787,000	948,000	1,068,000
Hemoglobin	7.55	8.14	8.08
Hematocrit	33.5	34.8	36.9

* Food efficiency: Total weight gain per food fed (dry weight)

** Performance index: (Average gain) (Food efficiency)

Nitrogen wastes

Partition of urinary nitrogen in carefully controlled studies is necessary to differentiate between the various forms of nitrogenous wastes. To our knowledge the partition of urinary nitrogenous wastes have not been fully established. Fresh water fish are known to excrete large amounts of urea and NH_3. Trimethylamine oxide has also been reported to be a significant form of excretory nitrogen. Differentiation between branchial and urinary excretions are incomplete. It was therefore decided to analyze separately each source of waste nitrogen.

The first step has been to collect undiluted samples of urine directly from the fish. This was done by cannulation of anesthetized trout which were then held partially immobilized in a plastic tube, see figure 7. (The technique of catheterization and holding chamber employed here are modifications of procedures developed by Dr. Robert Shiffman at Hanford, Washington.) A polyethylene catheter tube I.D. .045" x O.D. .062" was inserted into the bladder of trout weighing 450 to 550 gms. Approximately 45 mls of urine per day were collected for the first week. A 48-hour sample was analyzed in the Beckman Spinco amino acid analyzer by the procedure outlined for physiological fluids. The following list of compounds were tentatively identified:

High Amounts: Taurine, Urea, Ammonia, Histidine.

Moderate Amounts: Threonine, Serine, Asparagine, Glutamine, Glycine, Alanine, Leucine, Ornithine, Lysine, 3-methyl-histidine.

Low Amounts: Aspartic Acid, Creatinine, Glutamic Acid, Cystine, Cystathionine, Methionine, Isoleucine, Ethanolamine, Alpha-amino-n-butyric acid, Arginine.

Availability and utilization

This section of the laboratory program has in the past concentrated on determining a simple, reliable method for measuring protein quality. Research in this area by all groups is of extreme importance from the standpoint of nutrition as well as economics. Because salmon and trout are carnivorous animals the protein requirement is high. The economic pressures on our profession are increasing steadily as competition for protein by other industries continues to increase.

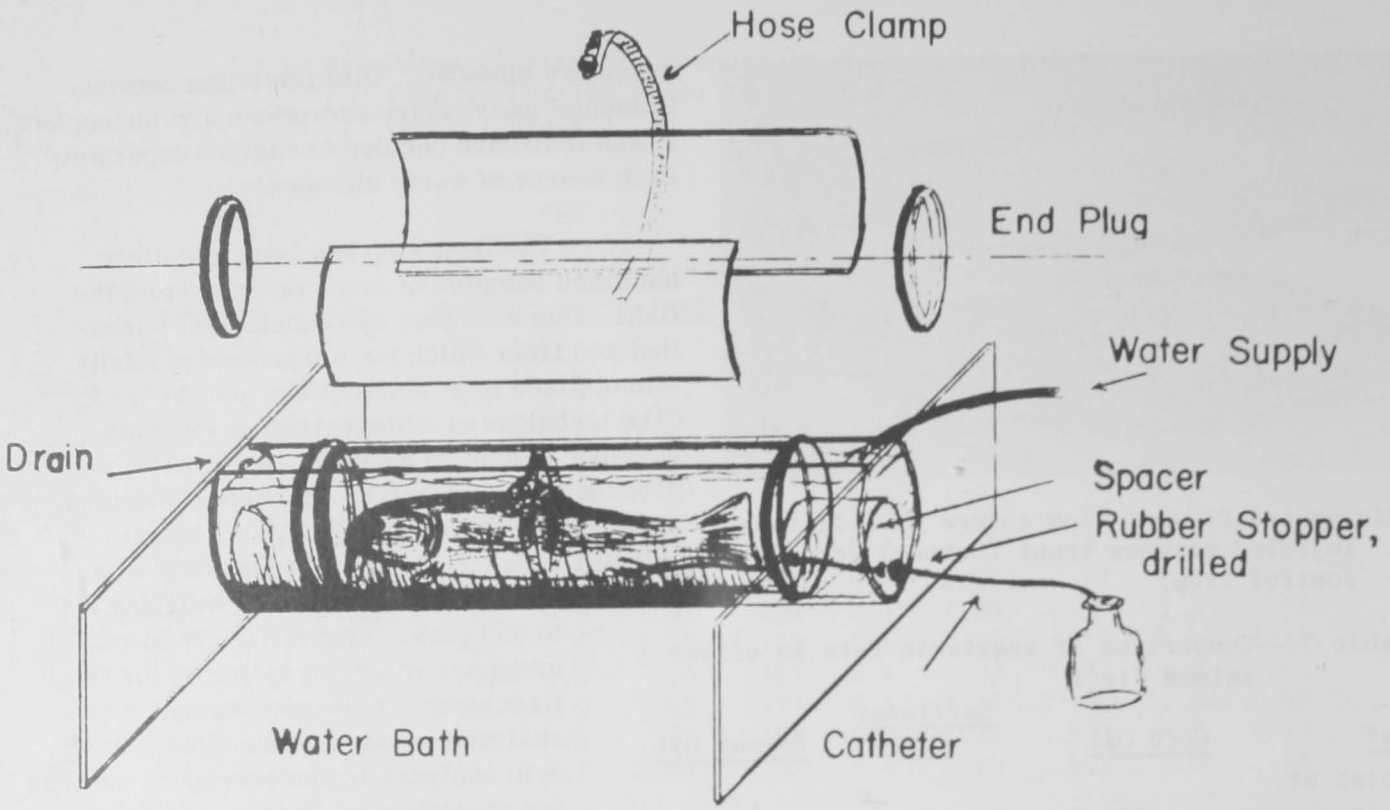

Figure 7:--The apparatus consists essentially of two pieces; an outer water bath and an inner, oval-shaped chamber holding the fish. The inner chamber consists of four pieces: the walls are sections cut from 6" plexiglass tubing. The end plugs are laminated pieces of $\frac{1}{4}$" plexiglass; one section conforms to the sides of the water bath to maintain the position of the holding chamber. An ordinary hose clamp holds the two sides together with further support from the end plugs. Sections from somewhat smaller tubing serve as spacers between the ends of the chamber and the water bath. Because the end plugs and spacers are removable a catheterized fish can be readily placed in, or removed from the chamber. The catheter tube is threaded through holes drilled in the end plugs and the water bath.

Probably the most frequently employed parameter for measuring relative nutritive value is the relationship between weight gain and quantity of protein fed, i.e. Protein Efficiency Ratio. Two valid criticisms can be made of this technique: 1. Weight gain is not necessarily protein, 2. Protein efficiency will vary with intake. However, even with these limitations the method provides useful data under controlled conditions. A requisite of protein efficiency studies is a uniform basic ration common to all diets.

The basal ration used in these feeding trials was compounded as follows:

Dextrin	50.0
Vitamins & Alpha-cellulose	18.0
Minerals	8.0
Corn oil & Cod liver oil	20.0
CMC	4.0
	100.0

To 50 grams of the ration were added levels of protein ranging from 10 to 45 percent (N X 6.25). The diet was supplemented with dextrin to 100. Moisture content was held at 50 percent (including H_2O of protein sources).

Three separate experiments were conducted. The first, at protein levels ranging from 15 to 45 percent indicated maximum efficiency between 15 and 20 percent protein. The second, at protein levels of 10, 15, and 20 percent indicated maximum efficiency of utilization around 20 percent. The third trial, at levels of 15, 20, and 25 percent completed this experiment. Again 20 percent levels of protein appeared to produce maximal efficiency of utilization. In all three experiments proximate analysis and hematological values were determined at the termination of the feeding trials.

The three feeding experiments are summarized in figure 8. Both averages values, as well as range in values are shown. Under the experimental conditions described, a level of 20 percent protein produced the maximum efficiency of utilization of protein with silver salmon. This is considerably higher than the generally accepted level of 9 to 12 percent protein found in rat experiments to produce maximum protein efficiency.

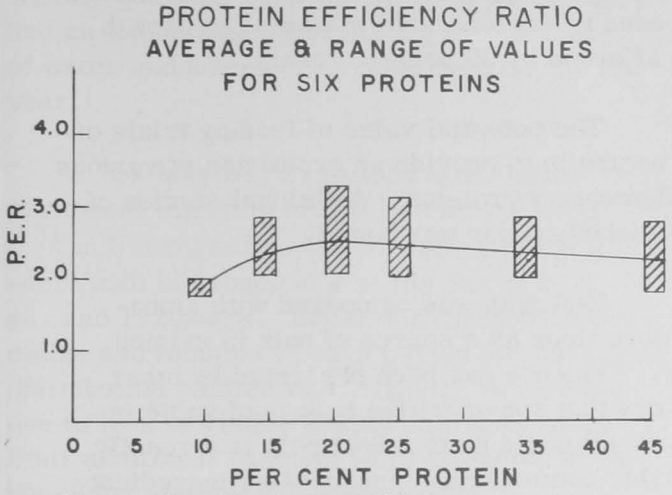

Figure 8:--Protein Efficiency Ratio

The data from P.E.R. #3 are typical and are summarized in table 8. It is of interest to note the high fat content of these fish which may indicate a pathological condition (although mortality was quite low), an extreme imbalance, or the ability of silver salmon to store fat. Hematological values were determined on a sample of 5 fish from each trough, or a total of 10 fish per protein level. These values are in general low but are not considered indicative of pathology. The low protein level, coupled with high carbohydrate may well explain the high fat content. In future trials a greater amount of non-nutritive bulk should be included to reduce the carbohydrate content.

Supplementary value of protein

It has been recognized for many years that the nutritive quality of combinations of protein frequently exceed their individual values. Thus the goal of many nutritionists is to compound a diet from poor and inexpensive proteins which is equal to one composed of relatively better, and more expensive proteins. The next

Table 8:--Protein Efficiency Ration No. 3

			Proximate Analysis					Hematology		
Protein	% in Diet	Ave. Wt.	P.E.R.	Prot.	Ash	Lipid	H_2O	R.B.C.	Hb.	Ht.
Turbot	10	1.209	1.80	50.0	7.8	41.8	75.6	968,000	7.9	32.2
"	15	1.788	2.64	45.2	7.6	45.8	74.7	839,000	7.5	28.2
"	20	2.248	2.71	43.9	7.7	46.5	74.4	1,004,500	7.8	28.9
Skim Milk	10	0.996	1.73	51.6	9.2	36.4	77.7	835,000	8.1	33.3
"	15	1.334	2.30	51.9	9.0	36.7	77.1	816,000	7.1	29.6
"	20	1.544	2.227	53.3	9.3	35.5	77.0	936,000	7.4	26.9
Salmon Meal	10	1.106	1.95	45.9	8.6	42.2	76.7	324,000	7.8	30.4
"	15	1.487	2.24	45.3	8.1	42.0	76.0	825,000	7.50	29.8
"	20	1.993	2.22	43.7	8.0	40.8	75.6	1,116,000	7.8	31.4
Salmon Eggs	10	1.095	1.80	49.3	7.8	40.8	75.9	1,003,000	7.9	34.0
"	15	1.644	2.64	44.9	6.7	47.4	---	1,018,000	7.8	30.5
"	20	2.550	3.42	44.3	6.7	45.8	71.9	946,000	8.0	33.3
"	--	---	---	58.1	10.0	32.8	80.0	**---	---	----
Cottonseed Meal	10	0.774	0.76	55.1	10.8	34.3	80.6	**---	---	----
"	15	0.818	0.50	54.0	10.3	34.7	80.7	---	---	----
	20	0.887	0.71	55.0	10.9	33.7	80.9	1,000,000	6.7	25.2

	% in Diet	Initial Wt.	Final Wt.	Prt.	Ash	Lipid	H_2O	R.B.C.	Hb	Ht.
Initial Fish	--	0.586	--	74.12	8.7	16.9	82.2	--	--	--
Non-protein	0	0.583	0.499	64.5	12.2	27.3	82.8	--	--	--
Salmon eggs	4	0.588	0.597	58.1	10.0	32.8	80.0	--	--	--

** Fish too small for analysis

feeding trial was designed originally to test protein quality; however, it was later realized that the experiment provided a better index of supplementary value of protein.

Due to the preliminary nature of this study proteins of fairly well known quality were selected. Gelatin, for example, is a poor protein due in part to the deficiency of tryptophan. Cottonseed meal, if of any value, should enhance growth if combined with a suitable reference protein. Salmon meal and salmon eggs had proved to be high quality proteins in previous studies. Casein is probably as uniform a protein as is available and has been used as a reference protein in previous studies at this station as well as in experiments with other animals. Casein at 4 levels (15 to 30 percent) was, therefore, taken for the reference, or control protein. Each of the test proteins at a 10 percent level were then combined with 20 percent casein. It was reasoned that growth beyond the 20 percent level of casein would be due to the test protein. Growth stimulation either in excess of, or less than, the 30 percent level of casein would provide an estimate of the supplementary value of the test protein in terms of a standard, or reference protein.

These diets were fed ad libitum to silver salmon for a period of 10 weeks. The results are summarized in table 9. A steady increment of gain corresponding to the protein level was noted in the casein control. Results with cottonseed meal were equivocal due to the high mortality.

Table 9:--Supplementary value of protein

DIET	PROTEIN %	GAIN (GMS)	AVE.WT. (GMS)	MORT. %	P.E.R.
CASEIN	15	71	1.27	7.6	1.39
"	20	113	1.57	7.7	2.06
"	25	147	1.81	7.6	1.91
"	30	193	2.09	4.3	2.02
CAS-C.S.M.	20-10	109	1.73	17.3	1.40
CAS-GEL.	20-10	163	1.90	5.7	2.05
CAS-SAL. M'L.	20-10	282	2.66	2.3	2.66
CAS-SAL. EGG	20-10	389	3.38	2.3	3.17

Considering average weight alone it would appear that cottonseed meal was of some supplementary value since the growth rate exceeded the 20 percent level of casein. From these data one would conclude that gelatin is superior to cottonseed meal, but inferior to casein because the growth rate exceeded cottonseed meal but fell short of the 30 percent level of casein. Both salmon meal and salmon eggs appear superior to casein since the growth rate exceeded by considerable margins the growth rate afforded by 30 percent casein.

The potential value of feeding trials of this nature is to provide an evaluation of various supplementary proteins. Additional studies of this method appear warranted.

Beet pulp was compared with alpha-cellulose flour as a source of bulk in salmon diets. Evidence has been presented by other workers that non-nutritive bulk is of value in certain salmonid diets. Beet pulp is a readily available commercially inexpensive ingredient, with other possible advantages since a low level protein (9.5 percent) and a high N.F.E. (50 percent not including fibre) are contained.

In this trial beet pulp and alpha-cellulose flour at 20 percent levels were incorporated into a common basal ration containing 20 percent solvent-extracted fish meal. The fish were fed on a paired basis for 8 weeks with beet pulp diet the factor governing intake. The results are summarized below:

Diet	Wt. gain	Prot. fed*	P.E.R.
Alpha-cellulose	182.9	661.5	2.98
Beet pulp	95.6	661.5	1.56

Diet	RBC	Hgb.	Hemat.
Alpha-cellulose	1,028,000	8.00	30.6
Beet pulp	984,000	7.91	31.2

* Does not include protein in beet pulp.

Beet pulp at this high level was non-toxic; however, appetite, growth, and protein efficiency were reduced. No major differences in hematological values were noted. Proximate analysis data are not complete. Future trials

at lower dietary levels appear warranted since no toxicity at this high level was observed.

Thyroid metabolism in salmon

Sixty-six migrating spring chinook salmon were obtained from the Bonneville Dam during the period of May 3-7, 1962. The salmon were held in cement raceways at the Willard National Fish Hatchery in water which had an iodine content of 0.25μg I^{127} per liter of water and a temperature of 6±1° C. for the year.

On May 24, 25 salmon after intraperitoneal injection of 100 μc carrier-free I^{131} in 0.1 ml saline (0.85 percent NaCl) were sacrificed in groups of 4 at the end of 4, 8, 24, 48, and 192 hours. Equal distribution between males and females at each period for the iodine distribution studies was virtually impossible due to lack of visible sexual characteristics. Four additional samples were collected at 48 hours for histological examination.

Twelve females examined had thyroidal iodine concentrations ranging from 15.6 to 126.0 μg I^{127} per thyroid region. The mean value of 49.37 μg I^{127} was not significantly higher when compared with the nine males which had a mean value of 28.02 μg I^{127} and a range from 12.5 to 73.9 μg I^{127} per thyroid region. Uptakes of the I^{131} in the thyroid region had attained a concentration of 11.68 percent of the injected dose at 192 hours. The slope of the uptake curve indicated, and T/S ratios confirmed that maximum uptake had not yet been attained and that an equilibrium had not been reached. On the other hand, muscle

$$\text{T/S ratio} = \frac{\text{concentration of Iodine/thyroid region}}{\text{concentration of Iodine/1 ml serum}}$$

samples taken adjacent to the dorsal fin attained a muscle maximum uptake of 7.4 percent of the injected dose at 8 hours and had decreased to 6 percent at 192 hours. In order that a closer approximation to equilibrium be attained in the thyroid region, it was concluded that the radioactive I^{125} isotope would be employed for all subsequent tests and that sampling period would be extended to 335 hours post injection.

This isotope has been found to be of value in extended time studies such as these because of its low energy radiation; making it highly desirable from the standpoints of safe handling and less damaging effects upon peripheral tissues in experimental animals. Its relatively long half life (60 days as compared to 8.3 days for I^{131}) not only permits experiments of longer duration but has enabled more definitive observations on long course studies.

Examination of the chinook salmon on August 2 showed all fish in good condition with few abrasions from the raceway. Little fungus was present, however, some copepods were evident on the branchii. Male sexual characteristics were slightly evident. Twenty animals were selected, attempting even distribution between males and females for intraperitoneal injection of approximately 500μc of carrier free I^{125} in 1/8 ml normal saline (850 mg NaCl per 100 ml).

A 2.5 fold increase in the maximum concentration of I^{125} in muscle was observed in August compared with the 6 hour period of May. This had decreased to 3.4 percent of the injected dose at 335 hours and was comparable to a projected estimate of muscular iodine concentration in the May group.

Correspondingly, the higher uptake rates of the muscle were also reflected in the percent of injected dose present in the thyroidal region of these adult salmon. The concentration rose rapidly to 1.3 percent of the injected dose at 72 hours and then assumed a more gradual rate of increase to 2 percent of the injected dose per thyroid region at 335 hours. A comparison of the I^{125} and I^{127} T/S ratios in August indicated that equilibrium of the radioactive material ranged from 41 percent to 98 percent completed in the individual fish at 335 hours post injection (table 10). The moribund condition of the eight remaining fish in September was noticeable in the I^{125} turnover. Whereas total muscular iodine concentrations remained comparable to the previous months at 0.470 μg I^{127} per 1 gram of muscle, the I^{125} uptake rate in the muscle had decreased to such an extent that at 72 hours after the injection maximum accumula-

Table 10:--Intrathyroidal iodine^{125} distribution

	August					September	
	Hours after injection						
	5½ hours	23 hours	71 hours	167 hours	335 hours	24 hours	72 hours
Origin	5.9%	13.1%	13.7%	15.2%	16.1%	12.8%	8.6%
MIT	13.6%	18.0%	20.2%	16.6%	22.8%	10.6%	21.3%
DIT	31.2%	42.4%	43.6%	31.9%	40.7%	30.1%	28.0%
Tx	0.0%	6.7%	10.3%	9.8%	6.0%	2.8%	10.0%
I-	49.2%	19.8%	12.2%	26.6%	14.4%	43.6%	32.2%

tion was not evident. Conversely, total thyroidal iodine content (20.1 µg I^{127} per thyroid region) was significantly different (0.005 level) from the previous months and uptakes at 72 hours had attained only 0.38 percent injected dose per thyroid region as compared to 1.3 percent at the same time in August.

The incorporation of I^{125} into iodinated amino acids in August and September revealed an apparent drop in the metabolic activity of the thyroid region which support the preceding observations of the moribund condition of the September samples.

Micro-iodine determination of the whole blood revealed significant differences (0.01 level of significance) in iodine content of both the males and the females between the May and August, and September groups. Examination of the blood fractions showed that these differences were not as great in the total serum portion as in the concentration of iodine that was organically bound. Micro-grams of iodine in blood fractions:

While the significant decreases in total iodine content of both the blood fractions and the thyroid region indicate that the total iodine pool of the fish is being depleted, the high proportion of total body tissue represented as muscle inhibits the detection of significant drops in muscular iodine concentration without larger portions of muscle being analyzed.

Studies of thyroid iodine distribution combining the use of I^{125} and I^{131} uptakes and I^{127} contents were made on two species of young salmonids. In all instances the fish were maintained in circulating water at 10±1° C. Part of the fish were fed a diet containing between 0.3 and 0.5 µg I per gram of dried diet (low iodine diet) and the other receiving the same feed but with added iodine to a level of 6 µg I per gram of dried diet (supplemented diet). Both diets as fed contained 66 percent water.

To obtain thyroidectomized animals, salmonids on the low iodine diet were submitted to 6 monthly intraperitoneal injections of 0.1

	Per 100 ml		Per 100 ml Serum						Per 100 ml Plasma			
	Whole Blood Total		Total		organ. bound Total		non-anionic Total		Total		non-anionic Total	
	female	male	female	male	female	male	female	male	female	male	female	male
May	12.28	12.41	26.73	33.99	16.46	23.68	15.36	25.72	17.07	21.03	11.93	12.55
Aug.	4.21	5.88	9.05	12.86	6.10	11.40	5.00	8.21	8.33	10.34	5.48	6.84
Sept.	5.54	2.57	14.21	10.98	7.23	6.74	6.46	6.48	12.52	9.06	6.33	4.85

normal saline containing approximately 100 μc of I^{131} as carrier-free iodide.

No significant variation in growth appeared between the thyroidectomized trout and their controls during the initial monthly weighings, however, by May, 1962 a slight decrease in weight of the thyroidectomized fish was evident (figure 9) and gross differences, manifested initially as a darkening of the skin pigmentation, were appearing (figure 10).

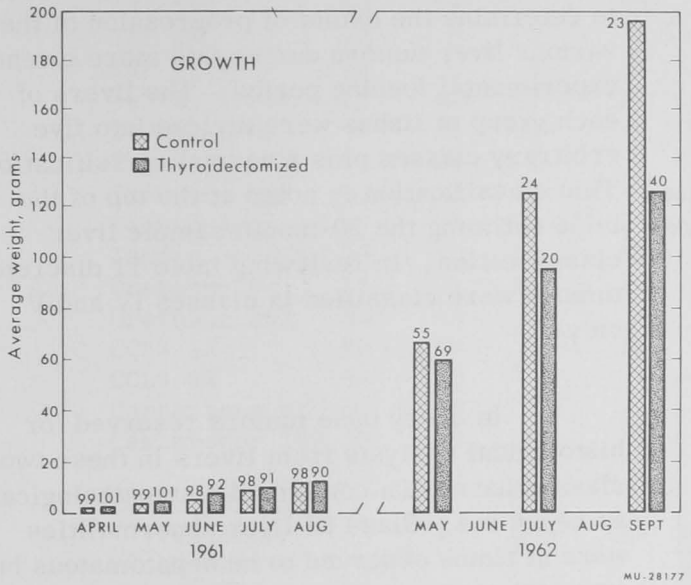

Figure 9:--Growth of thyroidectomized and control fish.

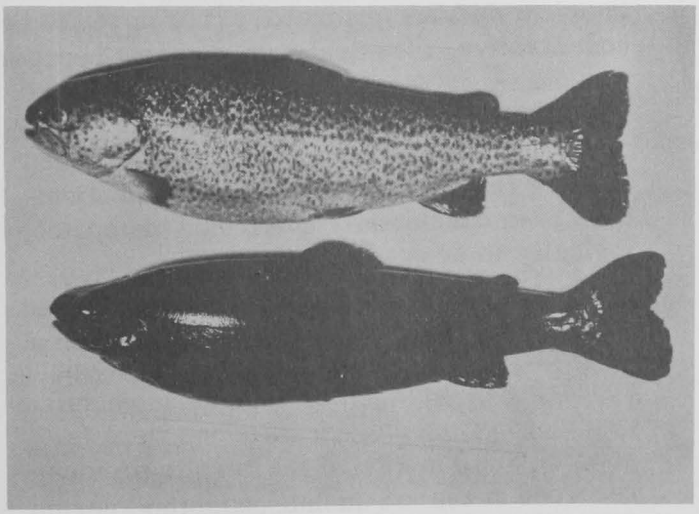

Figure 10:--Thyroidectomized and normal rainbow trout

By July significant differences in the size of the head were apparent and the fish had diminished sensitivity to external stimulation. The fish were also characterized by undeveloped gonads.

Micro-iodine determinations of total thyroidal iodine concentrations (0.5 μg I^{127}) less than the surrounding muscle tissue as compared with 3 μg I^{127} per thyroid region for low iodine diet fish and 26 μg I for the fish fed the supplemented iodide diet (figure 11).

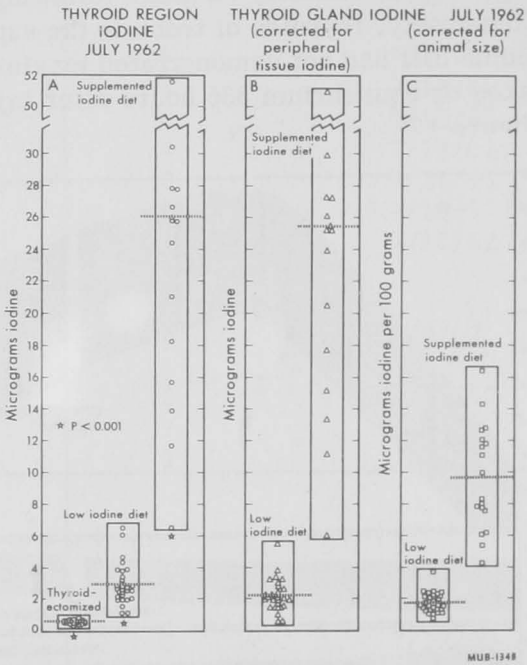

Figure 11:--Concentration of iodine in thyroid region.

Concurrent time studies conducted with the use of radioactive iodine tracers revealed thyroidectomized rainbow trout to have uptakes of approximately 2 percent at 24 hours. This value was not exceeded in any subsequent samplings. On chromatography of pancreatin-hydrolyzed thyroid regions, less than 3 percent of this iodine appeared organically bound, thus ruling out the possibility of metabolically effective thyroid tissue remnants.

Partially thyroidectomized young chinook salmon, as evidenced by histological examination, had 1 percent 24-hour uptakes and on chromatography of the thyroid hydrolyzates these animals had from 9 to 15 percent of this dose as intrathyroidal iodinated tyrosines. Trout on low supplemented iodine diets showed average 24-hour uptakes of 2 percent and 6.5 percent of the injected dose per thyroid region respectively. From 30 to 70 percent of the intrathyroidal I^{131} appeared as iodinated tyrosines in these animals. At 24 hours under these conditions, none of the radioactive material appeared to be incorporated in the tyroxine fraction.

The thyroids of trout on the low iodine diet reached maximum uptakes of 13 percent to 19 percent of the injected dose per thyroid region approximately 72 hours following injection. Conversely, thyroids of trout on the supplemented iodine diet had not demonstrated maximum uptakes or equilibrium 336 hours after injection (figure 12)

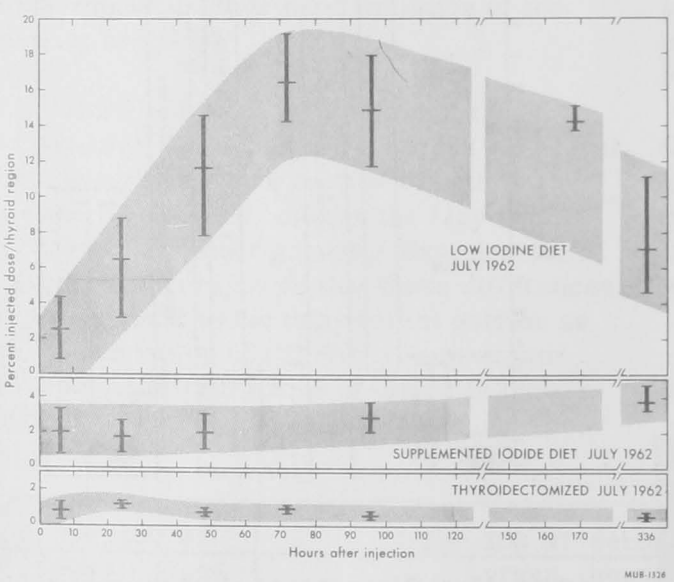

Figure 12:--Iodine uptake of thyroidectomized (lower) control (center) low iodine (upper) fed fish.

New groups of young fish are currently being prepared for the ensuing year. Five hundred chinook salmon have received 6 weekly injections of carrier-free 100 μc I^{131} and are awaiting histological confirmation of complete thyroidectomy. Five hundred each of rainbow trout and steelhead have received their first biweekly injection and will be completed by the end of February.

Induction of rainbow trout hepatoma with chemical carcinogens

Initial feeding trials utilizing fractions of the suspect commercial ration and known chemical carcinogens were terminated after approximately 20 months of feeding. At that time all animals were sacrificed with careful inspection of all external and internal organs for any indications of abnormalities. The various tumors observed in the liver and other organs were classified and compared with the previous inspection of these tissues in March, to determine the extent of progression of the various liver tumors during this more extended experimental feeding period. The livers of each group of fishes were divided into five arbitrary classes plus a normal classification. This classification is noted at the top of the table outlining the 20-month sample liver classification. In reviewing table 11 discrete tumors were classified in classes IV and V only.

In every case tumors reserved for histological analysis from livers in these two classes have been confirmed histopathologically as hepatoma. Class III liver abnormalities were at times observed to be hepatomatous but were not consistently so confirmed in every sample examined microscopically. In class I and II livers microscopic examination generally indicated some small necrotic areas from bacterial infection or other liver anomalies, and only rarely were small, basophilic staining, incipient nodules observed. Therefore, to be conservative, classical rainbow trout hepatoma was only suggested from gross observations from livers in class IV and V, and as stated previously, in every case thus far examined rainbow trout livers in these classifications have been consistently confirmed histopathologically as hepatoma.

As can be seen from table 11 the respective incidence of classical hepatoma appearing in diets fed these various chemical carcinogens and dietary fractions were in general confirmation of the results previously recorded at the 15-month sampling period. Obviously as the populations decreased and because of deliberately selected samples on surgical inspection the incidence might have

Table 11:--Hepatoma induction

N- Normal Liver
Class I - 1 to 5 spots remainder of liver normal
Class II - 5 to 20 spots remainder of liver normal
Class III - many spots with abnormal or swollen liver tissues
Class IV - discrete nodule with classical characteristics
Class V - advanced massive nodule, or nodules with metastastis

DIET	NO. EXAMINED	N	CLASS I	CLASS II	CLASS III	Classical Hepatoma CLASS IV	CLASS V	DATE TERMINATED
AAF 1/4	45	13	14	9	0	9		7/6/62
AAT 1/4	49	21	4	12	9	3		7/9/62
AAT 1X	43	5	10	2	3	21	1	7/6/62
ODT 1/16X	11	1	3	3	0	4		7/6/62
p-DAAB 1/16X	55	21	13	12	1	8		7/10/62
p-DAAB 1/4X	46	21	14	4	1	6		7/11/62
Thiourea 1X	42	21	5	3	1	11	1	7/11/62
Thiourea 4X	38	21	3	0	0	7		7/12/62
Tannic Acid 1X	48	5	12	19	6	6		7/12/62
Tannic Acid 4X	52	10	13	19	6	4		7/12/62
Urethane 4X	55	21	16	11	2	4		7/13/62
Urethane 16X	38	13	9	8	4	4		7/13/62
CCL4 1X	44	14	7	16	3	4		7/13/62
CCL4 4X	34	15	6	4	6	3		7/16/62
Carbarsone 4X	50	9	8	21	7	5		7/16/62
Carbarsone 16X	39	9	8	14	6	2		7/16/62
Carbohydrate Fraction	68	29	13	14	6	6		7/9/62
6% Extract Fat	11	2	3	1	0	5		7/17/62
Protein Fraction	41	18	6	11	4	2		7/3/62
Fat Fraction plus 6 mo. CTD plus prednisolone	11	4	3	2	2			7/11/62
Fat Fraction plus 6 mo. 70% CTD	15	2	0	5	3	5		7/17/62
Fat Fraction plus 6 mo. prednisolone	18	8	3	4	1	2		7/17/62
CTD	31	11	12	7	1			7/16/62

been expected to diminish because suspect samples were removed for subsequent histological analysis; however, the rate of appearance of hepatoma remained almost consistent in these surviving groups, with the exception of the additional nutritional treatments outlined below the graph line.

In those groups of fish fed the extract fat fraction for a period of up to 12 months the histopathologically confirmed incidence of grossly visible hepatoma was present at about 40 percent of the population. This large group was then sub-divided and then given various nutritional and hormone treatments. In that

small sub-group receiving the 70 percent regression diet plus prednisolone no grossly visible or histopathologically-confirmed hepatoma could be observed in any of the surviving samples. In that group fed the 70 percent protein regression diet only, grossly visible histopathologically-confirmed hepatoma appeared in five out of the fifteen surviving fish and histological analysis indicated some inhibition in hepatoma development. In that group continued on the extracted fat, but treated with prednisolone, terminal incidence of grossly visible hepatoma was noted in two of the 18 survivors. Histology indicated an alteration in cellular development of the hepatoma whereas that group continued on the 6 percent extracted fat has histopathologically-confirmed grossly visible hepatoma in five out of 11 of the survivors. These data indicated a pronounced and rather dramatic effect when prednisolone was administered to hepatoma carrying populations and the effect seemed to be improved when the high protein diet was also administered.

A brief summary of the terminal incidence of histopathologically-confirmed rainbow trout hepatoma after 20 months of challenging the various experimental groups with these chemical carcinogens at the levels previously described in the original experimental design were as follows:

AAF 1/4	9/45	Urethane 4X	5/55
AAT 1/4	3/49	Urethane 16X	4/38
AAT 1X	22/43	CCL4 1X	4/44
DDT 1/16X	4/11	CCL4 4X	3/34
p-DAAB 1/16X	8/55	Carbarsone 4X	5/50
p-DAAB 1/4X	6/46	Carbarsone 16X	2/39
Thiourea 4X	7/38	DMN 4X	38/46
Tannic Acid 1X	6/48	DMN 16X	14/41
Tannic Acid 4X	4/52	CTD	1?/300

In the CTD fed fish examined histopathologically, one probably incipient nodule was observed in over 300 liver samples examined of fish at least six months of age and extending to three-year-old fish fed for the complete growing period on the CTD ration. The older lots of these complete test diet fed fish are being continued and have been examined periodically at six-month intervals for any suspect area on the liver. To date none has been observed and, with the one exception mentioned, no grossly visible or histopathologically detected hepatoma nodule has been described in any of the samples examined. In those groups of fishes reared on the complete test diet in which the fat component was substituted with the extracted fat from the suspect commercial ration, an incidence of primary hepatoma appeared at about 40 to 50 percent of the population and this incidence can be extended to about 60 percent of the population when smaller histopathologically detected nodules are included in the samples examined. In contrast when fish with grossly visible hepatoma nodules were removed from the original diet and were given a nutritional treatment plus prednisolone, no grossly visible hepatoma nodules could be observed. The small group of fish given this particular diet treatment alone, seemed to have altered cellular characteristics of hepatomatous areas of the liver, but the incidence of hepatoma was only slightly affected. Some slight influence of the hormone itself was also indicated histologically upon terminal examination of the small group of fish receiving fat plus prednisolone.

Special glassware including a large cyclone evaporator and equipment for more refined chromatographic separation of the neutral lipid and phospho lipid fractions in the extracted commercial fat for the current studies has been received. Preliminary test columns on silicic acid and powdered glass has enabled the separation of the neutral lipid fraction into six groups of compounds (fig. 13). These

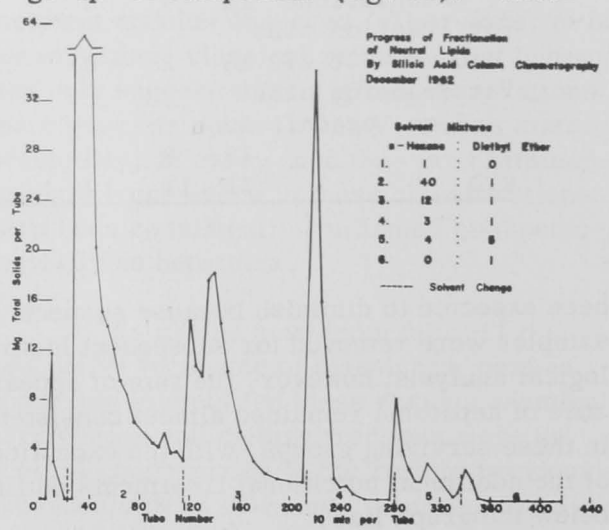

Figure 13:--Progress of fractionation of neutral lipids by silicic acid chromatography, December 1962.

groups will be tested for carcinogenic activity in new feeding trials scheduled to begin in February.

The second induction study of rainbow trout hepatoma with chemical carcinogens

The second induction study has been in progress for 10 months. Using the same chemical carcinogens previously found effective in the appearance of hepatoma, paired groups of fish were fed these compounds at the minimal level and the correspondingly lower level of the particular chemical found to be effective in the production of hepatoma in the first induction experiment. After feeding paired groups of fish these rations for six months, ten representative samples were removed from each group of fish for histological analysis and the paired lots combined into one larger lot. This larger lot was then moved to six-foot circular tanks for further testing. Because of tank space limitation and because little or no loss was experienced over a six- to seven-month feeding period duplicate tanks of the various groups of fish could not be incorporated with equipment available at the Hagerman Station. The livers and other internal organs were closely examined for appearance of any abnormalities and all ten samples selected were preserved for histological analysis.

Histological examinations have been negative, thus far, for hepatoma in the following:

2 acetylaminofluorine
aminotriazole
carbontetrachloride
DDT
p-dimethylaminoazobenzene
Dimethylnitrosamine
Tannic Acid
Thiourea
Urethane

However the following abnormal pathologies have been noted:

1. Aminoazotoluene:--microscopic hepatomas in two samples

2. Carbarsone:--incipient nodules in two samples

3. Thioacetamide:--incipient microhepatomas or diffuse Cholangiomas in three samples.

Biopsies and tumor transplants

In the terminal experiments of the first phase investigation of biopsy techniques and transfer techniques, tissues were implanted into cardiac chamber, peritoneal cavity, liver, stomach wall, pyloric caeca, spleen, kidney, testes, ovary, eye chamber, intra-muscular area, and subcutaneous facia, respectively. The individual fish were triple matched with serum prepared to identify donors and acceptors respectively prior to the transplant. Recipients were fish which had been fed only the laboratory complete test diet for their entire life. Histopathologically-confirmed hepatomatous tissue was observed in recipients for tissue transplanted into the liver, the stomach wall, the pyloric caeca, the testes, subcutaneous facia, intra-muscular area, cardiac chamber and peritoneal cavity. These experiments have now been terminated with the general indication that hepatoma tissue can be implanted or transplanted between individuals when they are closely matched genetically.

Cooperative research

Acetylaminofluorine intermediates will be studied by Dr. James Miller at the University of Wisconsin. With the apparatus outlined in figure 7, rainbow trout were cannulated and were force fed with 10 mg doses of N-2fluorenacetamide contained in 5.0 gms of complete test diet. Urine was collected for 24 hours prior to the challenge with the carcinogen and for 72 hours after the challenge. The urine was preserved with toluene and was shipped to Dr. Miller for determination of the 2-hydroxy derivative. When the assays are complete further information will be assembled on the pathways of this particular carcinogen in fish tissues.

Labeled dimethylnitrosamine was prepared by Dr. Peter Magee in England and was

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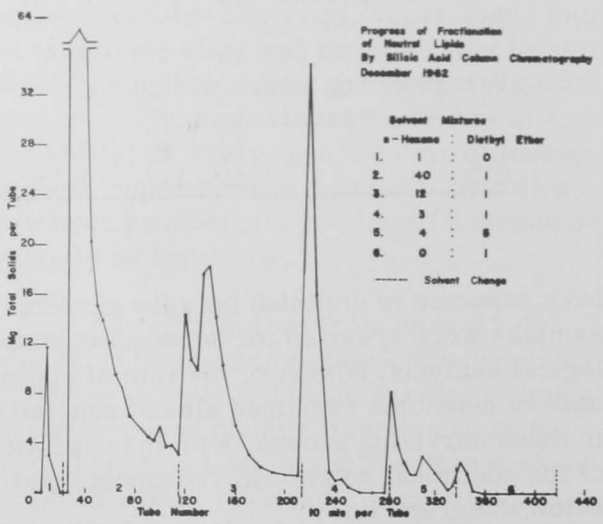

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Labeled dimethylnitrosamine was prepared by Dr. Peter Magee in England and was

shipped to the laboratory for subsequent challenge to six large rainbow trout held in constant flowing 10° C. water. After challenge by force feeding the labeled compound incorporated in the complete test diet, two fish samples were sacrificed and blood serum, liver homogenates, kidney homogenates, muscle homogenates and heart tissues were prepared for shipment to Dr. Philippe Shubik at the University of Chicago. Personnel at the University of Chicago further prepared the tissues isolating potential intermediate system and conducted the preliminary counting. The final preparations were then shipped to Dr. Magee for ultimate analysis. This study should also indicate the particular pathways followed in the detoxification of dimethylnitrosamine and may yield a clue as to the particular moieties responsible for interfering with normal cellular metabolism and allowing neoplastic growth to initiate.

Polycyclic polymerized lipids have been received from Dr. Fred Kummerow at the University of Illinois for subsequent incorporation into the diet during the third induction studies scheduled for February. This polymerized material was isolated by Dr. Kummerow from heated fats and is the same material reportedly responsible for the threefold increase in sensitivity to carcinogen challenge by 2-AAF in rat hepatoma study. This material will be incorporated into the complete test diet as a portion of the lipid fraction and animals receiving this material at three different levels will be challenged with AAF and dimethylnitrosamine to measure the effect of heated polymerized fats on carcinogen dosage levels effective for the appearance of hepatoma.

A cooperative research venture with Dr. Nevin Scrimshaw and Dr. Gerald Wogan at Massachusetts Institute of Technology and the laboratory testing the effect of Aspergillus flavus contaminated meals on rainbow trout has been organized and the study will be initiated in February. Dr. Wogan has prepared liquid media concentrates which have extremely high toxicity in duckling assays and reportedly induced hepatoma in rats at a low concentration in the diet. This material has been further fractionated into reproducible migrating compounds on thin layer chromatography plates with a tenfold increase in toxicity on duckling bioassay. The concentrate material and at least two of the potent fractions have been shipped from MIT to be incorporated into initial feeding experiments in February. Additional concentrates from flavus contaminated peanut meal will be obtained for confirmatory tests. The duckling assays will be conducted at MIT and the trout bioassays will be completed at the Hagerman station. Total lipid concentrate, neutral lipid fraction and phospho lipid fraction have been sent to MIT for incorporation in their current tests of toxicity of these compounds on experimental animals and on characterization of the particular moieties present by thin layer chromatography and physical biochemical analysis.

Total lipid concentrate, neutral lipid fraction and phospho lipid fraction were shipped to Dr. Carrol Smith at Orlando, Florida, for studies on potential toxicity and tumor induction with experimental fly populations. Recently a report was received from Dr. Smith that these compounds were found ineffective and carried little residual toxicity to experimental fly populations at the levels used in their challenge. This particular cooperative research venture was therefore cancelled pending further study by Dr. Smith.

Potential vitamin status experiments have been organized between Dr. Myron Brin, Upstate New York Medical Center, and the laboratory. Potential thiamin status of the fish will be measured by adapting specific transketolase activity measurements and pyridoxine status will be measured by erythrocyte transaminase activity. In a preliminary experiment exploring the reliability of erythrocyte transketolase as a biological assay tool for physiological thiamin status in the animal, a group of rainbow trout has been made thiamin deficient by deleting this vitamin from the complete test diet. Approximately 60 fish are becoming thiamin deficient. As soon as 10 percent of the population dies exhibiting the specific thiamin deficiency syndrome a portion of the deficient population will be injected with various thiamin dosages, and blood serum levels following administration of the missing vitamin will be assayed by the technique Dr. Brin has developed for erythrocyte transketolase activity.

The specific activity of this enzyme will also be compared with control fish and from fish at various stages of thiamin deficiency. In the event that other specific tissue transketolases may be more indicative in trout for thiamin status estimates, smooth muscle tissue, heart, liver and kidney tissue will also be included in the transketolase specific activity assays. As the techniques become perfected for measuring thiamin status, pyridoxine status will be investigated by measurement of specific activity of tissue transaminases, also following the techniques developed by Dr. Brin. It is anticipated that extremely interesting results will be obtained from the transaminase activity tests because of the high protein requirement of fish and the correspondingly higher demands for pyridoxine, an essential in the co-enzyme for transaminase reactions. After preliminary experiments are completed, it is anticipated that Dr. Brin will visit the laboratory and complete the development of clinical assay techniques for measurement of B vitamin status in trout and salmon populations.

In a cooperative venture between the laboratory and the Samuel Noble Research Foundation, Ardmore, Oklahoma, Dr. Paul Kruse has received and prepared trout and salmon sperm for assay and separation of arginylarginine. Sperm has been obtained and shipped to Ardmore for the preliminary experiment. As these techniques become perfected Dr. Kruse anticipates shipping to the laboratory radioactive labeled arginine which will be incorporated in the diet of sexually maturing rainbow trout. Hopefully the arginine will be incorporated into arginylarginine in the salmine component of the sperm being synthesized rapidly at this state in the life cycle, and upon stimulus with chorionic gonadotropins, the sperm obtained should contain radioactive labeled arginylarginine. This particular material will then be used in neoplastic tissue culture experiments to measure the effect of this marked intermediate in neoplastic cell synthesis. Radioactive labeled arginylarginine is difficult to obtain by normal chemical synthesis techniques. Biologically active radioactive labeled arginylarginine should be obtained through this cooperative research venture allowing the fish to synthesize the material desired. The specific role and function of this di-peptide in stimulation of abnormal cell growth should add to our information on the basic intermediary metabolism functions in normal and aberrant cell metabolism and may perhaps yield a key to the better understanding of stimulated cell metabolism into cancerous growth.

Red blood cells were obtained from rainbow trout, silver salmon, and chinook salmon for subsequent hemoglobin analysis by Dr. Austen Riggs at the University of Texas, at Austin. Erythrocytes were carefully washed three times in Alsever's solution and were carefully packaged for shipment to the University of Texas by air. All shipments were received in good condition and have subsequently been processed for analysis. A comparison of the different types of hemoglobin obtained from the different species of fish will add to the characterization of hemoglobin from salmonids. Dr. Riggs will be corresponding with Dr. Donald Buhler who recently published a paper on the multiple hemoglobins in salmon and trout, and in addition will be corresponding and working with Dr. Hugh Tarr's group at the Fishery Research Board of Canada, located at the University of British Columbia where they are currently involved in sockeye salmon and pink salmon hemoglobin studies.

Attempts to classify the indispensable amino acids for channel catfish in a cooperative research venture between the laboratory and the Southeastern Fish Cultural Laboratory have not met with outstanding success. Little or no absorption of the amino acid mixtures administered to the channel catfish has been observed and further work must be planned in this area before standard test diets acceptable to the catfish and carrying positive experimental control of particular amino acids in question can be formulated to carry on this study. Details of the problems encountered and the results obtained to date will be found in the report from Southeastern Fish Cultural Laboratory.

Dr. Ronald E. Chance and Dr. Edwin T. Mertz at Purdue University have now completed the amino acid patterns found in the

serum of actively feeding oceanic chinook salmon and from terminal migrating chinook salmon. Trout serum aminograms have also been completed at the laboratory yielding much informative material on estimation of the quantitative amino acid requirements of salmon and trout through blood serum amino acid patterns. Profound differences were observed between the migrating chinook salmon serum amino acid patterns and those from the actively feeding and partially starved chinook salmon. Similar differences, although not as profound, were observed in the rainbow trout patterns. Some estimates of the amino acid requirements can be obtained from analysis of blood serum amino acid patterns obtained after a critical starvation period where major influences from ingested amino acids do not interfere with the essential blood serum amino acid pattern for maximum protein synthesis.

In a similar study, aminogram preparations were made from flathead catfish, blue catfish and channel catfish for subsequent amino acid assay. Analysis of the patterns obtained from these preparations will not be completed until more work is done with salmon and trout blood serum patterns. It is anticipated that a graduate student thesis program will be organized for the coming summer to perfect this technique and enable the investigator to estimate the qualitative and quantitative indispensable amino acid requirements of a number of fishes from analysis of the blood serum amino acid patterns obtained after critical starvation periods. The results obtained to date are extremely interesting but are not complete enough to include in tabular form because of the inconsistencies observed at different starvation periods and different dietary history. Undoubtedly this technique when perfected will offer an important clinical tool for measurement of the protein and amino acid status of animal populations.

Montana and Nevada cutthroat trout test diet experiments

Early in July, Montana and Nevada cutthroat trout eggs were obtained, hatched, and the fry fed six different nutritional treatments plus a commercial diet in attempts to develop a test diet satisfactory for cutthroat trout nutrition experiments. The various treatments included: 60 percent casein diet; 45 percent casein diet; the standard complete test diet; at one and twofold vitamin complements respectively, plus a commercially available diet. A profound difference in the response between cutthroat trout originating in Nevada from those obtained in Montana was observed. Little or no growth was obtained on the various casein diets in the Nevada cutthroat groups after six weeks of feeding. Mortality became excessive and this part of the experiment was terminated. Those groups of Nevada cutthroat maintained on the complete test diet at the 1X vitamin level continued to grow at a fairly slow rate. After 16 weeks of feeding the Nevada cutthroat trout were approximately 60 percent of the size of the Montana cutthroat trout when the complete test diet was fed at twofold vitamin supplement. Mortality was fairly low and perhaps this particular diet could be used for some preliminary nutritional estimates.

With the Montana cutthroat trout reasonably good growth was obtained when a 60 percent casein diet was fed with a double vitamin complement or when a 45 percent casein diet was used. Response with the complete test diet was of the order of magnitude of that obtained with the Nevada cutthroat trout and was considerably less than that obtained from the commercial ration. Most experimental histology samples have not been analyzed and may perhaps yield clues as to the inconsistency of the response between groups and for the unaccountably high mortality periodically encountered with different lots of fish exposed to these various diet treatments. Further work with cutthroat trout experimental test rations will be conducted in an attempt to clarify these inconsistencies.

Amino acid content and chemical score

Amino acid analysis has for some time been a major area of research in this laboratory. With the advent of automated amino acid analysis great strides have been made in this field. In the past year a series of diets and individual diet ingredients have been analyzed. In this

period a total of 196 runs have been made including calibration checks and blood aminograms.

A major objective of this program has been to compile a catalog of diets and components in which the complete amino acid spectrum is defined. At this point over 30 samples of commercial, hatchery, experimental diets, and individual diet ingredients have been assembled. To make this information of greater utility, chemical scores have been calculated using the classical method of Oser. The values listed for whole egg protein are those of Orr and Watts and were determined by microbiological assay. To establish a better correlation of their data, a sample of whole egg protein (commercially available) was included. These data are shown in table 12.

Table 12:--Chemical score of fish diet ingredients.

Dietary Ingredients	Chemical Score[2/]	Limiting Amino Acid[3/]	Percent Protein
Whole egg - Orr & Watts[1/]	100	None	
Whole egg - Commercial	92.9	Isoleucine	45.9
Salmon egg	93.9	Methionine	
Past. Salmon Products[4/]	85.6	Isoleucine	
Autolyzed Salmon Products	84.9	Isoleucine	
Casein	83.7	Arginine	93.0
Skim Milk	82.7	Arginine	33.7
Salmon Viscera	81.5	Isoleucine	
Herring	81.4	Isoleucine	
Herring Meal	80.8	Isoleucine	74.7
Turbot	77.9	Valine	
Salmon Flesh	77.8	Isoleucine	
Drackett (soybean protein)	76.8	Methionine	84.4
Salmon Meal	70.5	Isoleucine	66.9
Sesame Oil Meal	67.6	Lysine	43.4
Wheat Germ Meal	66.6	Methionine	27.5
Brewers Yeast	66.2	Methionine	49.0
Distillers Solubles	66.1	Lysine	27.9
Cottonseed Meal	63.7	Methionine	44.6
Wheat Middlings	58.3	Isoleucine	17.8
Shrimp Meal	54.9	Methionine	38.0
Crab Solubles	50.3	Isoleucine	52.0
Commercial Diets			
#1	80.4	Isoleucine	39.7
#2	79.2	Isoleucine	
#3	75.4	Isoleucine	
#4	74.7	Methionine	39.7
#5	72.2	Isoleucine	43.1
#6	70.6	Isoleucine	
Experimental Diet	77.4	Isoleucine	54.7
O.M.P.	72.4	Isoleucine	36.0

1/. Orr, M. L. and Watt, B. K. (1957) Home Econ. Research Reports No. 4.
2/. Tryptophan was excluded from the chemical score since it is destroyed in the HCL hydrolysis procedure.
3/. Basis of whole egg protein.
4/. Consists of equal parts pasteurized salmon flesh, salmon viscera and salmon eggs.

HISTOPATHOLOGY

Hepatoma induction

The first series of induction experiments, begun in October 1960, included 13 classical rodent carcinogens each fed at 5 different levels. Hepatoma was found in rainbow trout fed all but aminotriazole, and thioacetamide, the two which were started about 8 months after the others. However, one liver with an incipient adenocarcinoma was found in a trout fed thioacetamide for 15 months. The following diets showed histopathologically-confirmed hepatoma in varying incidence although final calculations of incidences versus level of carcinogen fed are as yet incomplete:

1. 2 acetylaminofluorine: microscopic neoplasm after 11 months and sarcomatous hepatoma after 21 months, figure 14.

2. Aminoazotoluene: trabecular and cholangiomatous hepatomas, gross to microscopic size after 10 months, figures 15 and 16 and in one case a spleen with fatty necrosis and fibrosis, figure 17.

3. Carbarsone: trabecular hepatomas of minute to microscopic size after 20 months.

4. Carbon tetrachloride: microscopic trabecular hepatoma after 15 months.

5. DDT: microscopic trabecular hepatoma after 11 months.

6. Paradimethylaminoazobenzene: microscopic trabecular hepatoma after 11 months.

7. Diethylstilbestrol: microscopic trabecular and adenocarcinomatous hepatoma after only 7 months.

8. Dimethylnitrosamine: typical minute trabecular hepatoma, figure 18, after 11 months and advanced gross degenerative and sometimes hemorrhagic neoplasms which measured up to 2.5 cm in greatest diameter figures 6, 7, and 8. There was an incidence of 40 percent hepatoma at the 4X level and of 100 percent hepatoma at the 16X level based on gross diagnosis at autopsy. All of the reportedly hepatomatous samples when examined microscopically were hepatomatous. Three unusual renal neoplasms were also found in these samples and were all diagnosed tentatively as adenosarcomas.

9. Tannic acid: microscopic adenocarcinoma after 12 months and typical trabecular hepatomas in later samplings up to 21 months. A few of these were grossly recognizable.

10. Thioacetamide: incipient adenocarcinoma after 15 months.

11. Thiourea: hepatoma some of which were adenocarcinomatous and measured up to 4 mm in diameter.

12. Urethane: somewhat atypical more or less acidophilic trabecular hepatoma all of microscopic size after 12 months.

Samples from fish fed various extracts of a suspect pelleted feed showed:

Fat: incipient hepatoma after seven months and hepatoma grossly visible after 12 months.

Protein: one gross neoplasm confirmed microscopically found after 17 months on the diet.

Carbohydrate: small hepatoma nodules after 12 months.

Complete test diet (50 percent protein): one microscopic suspect incipient hepatoma after 10 months out of nearly 300 trout livers sampled microscopically.

A second confirmatory series of similar but slightly lower level carcinogen feeding experiments was begun in February 1962 and early samples have shown the following (after approximately 8 months):

1. Aminoazotoluene: microscopic hepatoma in two samples.

2. Carbarsone: incipient micro-hepatoma in two samples.

3. Thioacetamide: incipient micro-hepatoma or diffuse cholanginomas in three samples.

4. Fat extract: one microscopic hepatoma and seven incipient micro-hepatomas.

5. Neutral lipid extract: one micro-hepatoma and one incipient micro-hepatoma.

6. Phospholipid extract: no confirmed hepatoma.

7. Protein: one suspect incipient micro-hepatoma.

8. Carbohydrate: one suspect incipient micro-hepatoma.

9. Stripped herring oil: one micro-adenocarcinoma (suspect).

Note: Except for dimethylnitrosamine, which has produced high incidences of multiple nodular hepatomas to advanced stages of degeneration, the classical carcinogens fed for hepatoma induction have produced mostly small or microscopic hepatomas, which would have doubtless enlarged had the trout been permitted to survive.

Livers from 15 small catfish reared on a suspect pelleted ration at the Southeastern Fish Cultural Laboratory, Marion, Alabama, were processed and examined for possible hepatoma but none was found. These livers were of histological interest because of their characteristic hepato-pancreas-sheaths of pancreas tissue surrounding the portal veins and their branches within the liver.

Thyroid histology

Thyroid tissue from 11 rainbow trout from the aminotriazole 1X hepatoma induction experiments was examined. Stained sections showed considerable thyroid hyperplasia characterized by dense masses of tissue with elongated follicular cells, follicles with invaginated or slightly papillated mucosa but with little or no colloid in the lumens. The massive nature of these thyroid aggregations strongly suggest incipient adenoma, a benign tumor. Besides this there was some leucocytic infiltration in the interfollicular connective tissue and adjacent fat and subcutaneous connective tissue. Normal rainbow trout thyroid is more diffuse, the follicules more spheroid and the lumens contain acidophilic colloid. This strongly suggests that the administration of aminotriazole has thus far been essentially negative with only an occasional liver section suggesting prehepatomatous change.

Hemopoietic cytology

Incident to studies on salmonid hemopoietic tissues an effort to find the best method for determining the mitotic index of developing blood cells resulted in the use of impression films stained with Wright's followed by Giemsa's blood stain. Counts of some 4,000 immature red and white cells gave a mitotic index of approximately 3.5 percent (3.5 mitoses per 100 cells considered capable of mitosis). This figure remains tentative and further work with both normal and anemic salmonids is contemplated for 1963.

Histology

Library research on the normal histology of the salmonid alimentary tract was nearly completed. Several references were read and an outline prepared. Writing was begun on this phase of the project and some photomicrographs suitable for pharynx necessary for this project are in preparation in the histology laboratory.

Infectious diseases

Trichodina discoidea, figure 22, (dark phase photomicrograph) was diagnosed in a population of control rainbow trout fed a complete test diet. The large protozoan parasites were densely aggregated on the skin and unidentified bacteria appeared to have caused a secondary infection. The eight- to nine-inch trout were dying at an alarming rate prior to discovery of the parasites and bacteria.

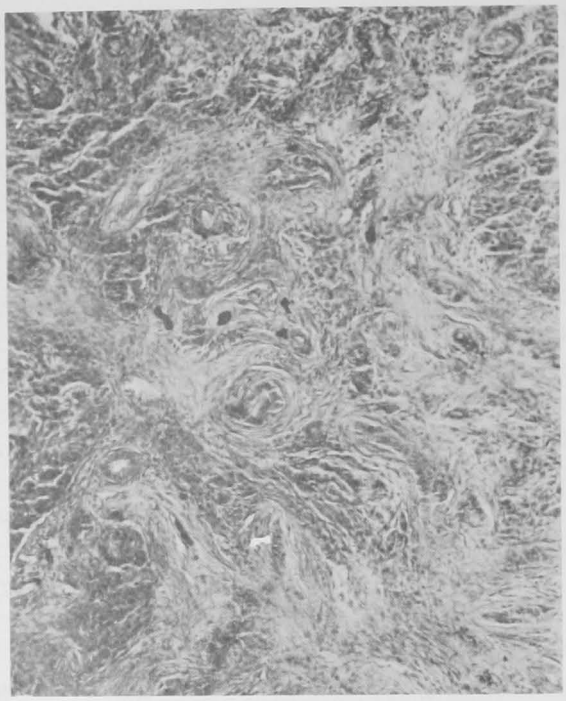


Figure 14:--Rare sarcomatous pattern of adenocarcinoma of rainbow trout liver. The diet was acetylaminofluorine, $\frac{1}{4}$ X level, for 21 months. Note whorls and strands of spindled liver cells alternating with cords and islands of more or less unaltered liver cells. X 100

Figure 15:--Dark basophilic invasive hepatoma with vacuolated, more or less necrotic focus (top center). Altered liver cells with small cytoplasmic vacuoles surround the neoplastic and necrotic areas. Trout fed aminoazotoluene, 1X level, for 11 months. X 100

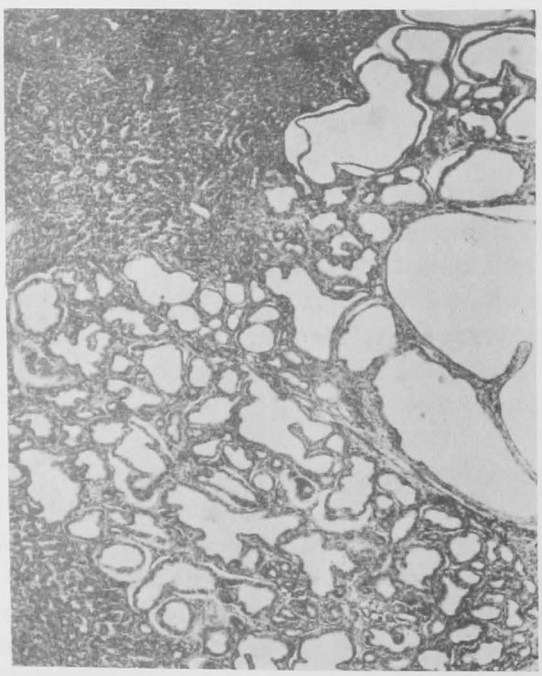


Figure 16:--Adenocarcinoma of rainbow trout liver showing moderate development of multilocular cysts. Trout was fed aminoazotoluene 1X level, for 21 months. X 100

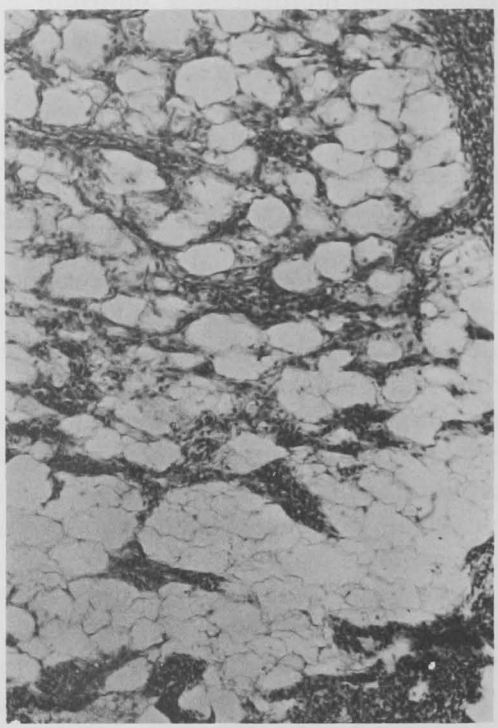


Figure 17:--Spleen from trout fed aminoazotoluene 1/16X level for 12 months showing fatty change and increased fibrosis. X 100

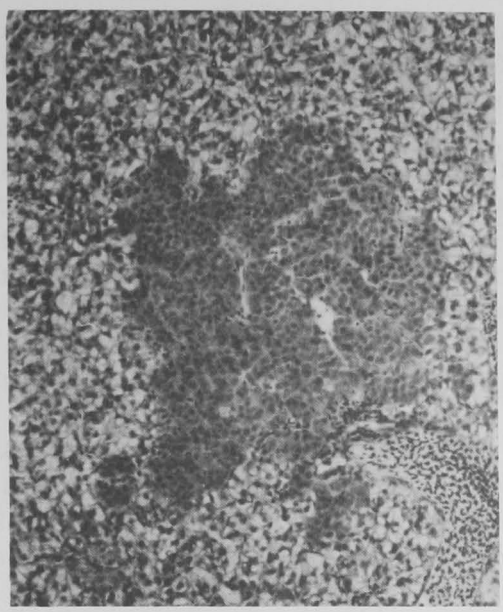


Figure 18:--Invasive early liver cell carcinoma surrounded with heavily vacuolated liver cells. Trout fed dimethylnitrosamine, 16X level, for 11 months. X 100

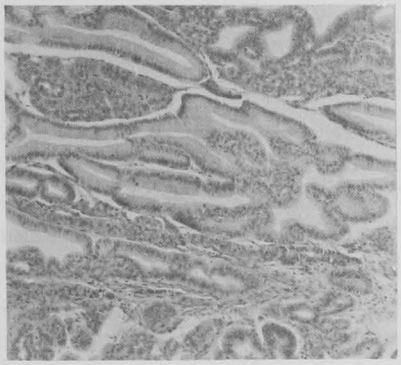


Figure 20:--Hepatoma of rainbow trout fed dimethylnitrosamine, 16X level, for 15 months showing extensive adenocarcinoma with mitotic figures common. Note unusually tall columnar epthelial cells lining the elongated gland-like formations. X 100

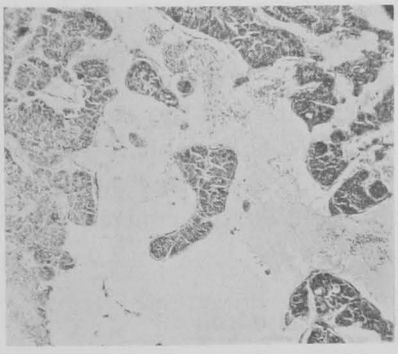


Figure 21:--Hepatoma of rainbow trout fed dimethylnitrosamine, 16 X level for 4 months showing extensive degeneration following multiple cyst formation amongst the much altered and widened liver-cell cords. X 100

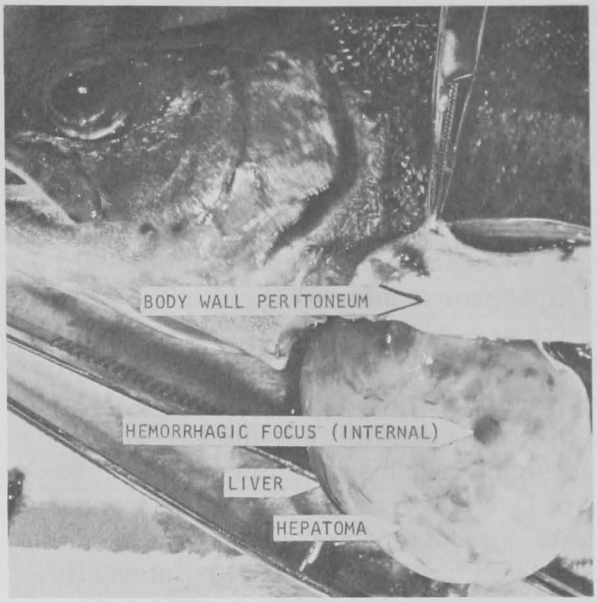


Figure 19:--Nearly two-year-old rainbow fed dimethylnitrosamine, 16X for 21 months with 20 x 30 mm, eight gm hepatomatous liver of greenish-yellow color and of soft texture. Ventral surface appeared to be about 15 percent hemorrhagic. The fish remained in "good" physical condition until autopsy. The spleen and kidney were normal. Upper arrow--body wall peritoneum; lower arrow--hepatoma proper; long middle arrow--hemorrhagic focus and short middle arrow--relatively unaltered liver. Approximately X 2

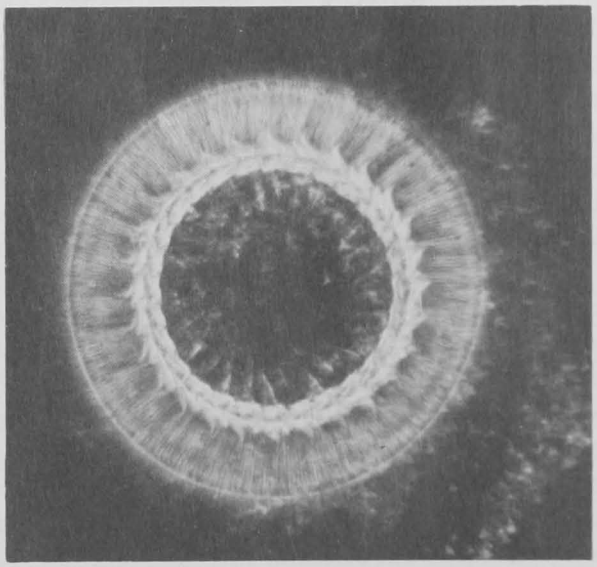


Figure 22:--Trichodina discoidea. Living animal photographed with phase microscopy Heavy infestations of this protozoan were found on the skin of 2-year-old rainbow trout fed a complete test diet approximately two years and held as controls for the hepatoma induction experiments. X 375

Subsequently the condition cleared up following prompt treatment with formaldehyde. The source of the Trichodina was probably water from the Little White Salmon River.

Dactylogyrus sp., a gill fluke, very difficult to photograph, was identified in November from a small population of sword-tails (Xiphophorus helleri), held in the Laboratory's tropical aquarium. The "four-eyed" flukes were numerous to abundant on the gills of sick and moribund fish which had been propagated in our aquarium for some five years without significant losses from disease. Because of Dactylogyrus and possible secondary invaders, although bacteria could not be demonstrated either externally or internally, this population was almost destroyed within a short time and before effective chemotherapeutic measures could be taken to save them. Only four newly-hatched young were saved from this gross morbundity. It is believed likely that a virus infection may have been superimposed upon that of Dactylogyrus in this case. All tanks in this aquarium have since been washed and disinfected in anticipation of the introduction of new tropical fish stocks.

Microslide and photographic libraries

Approximately 6,200 stained, labeled, catalogued, read and filed microslides of fish tissues have been added to our micro-slide library during the year. Photomicrographs and some gross photomacrographs totaling 1,063 black and white and 420 Ectachrome pictures have been added to our photo-library in 1962.

Hematological changes in fall chinook salmon blood

Possible hematological changes in fall chinook salmon blood during the adult stage from the actively feeding salt water fish to the time of sexual maturity, have been studied. Blood samples were taken at sea from fish caught by hook and line. Samples were taken from fish caught in gill nets in the brackish water at the mouth of the Columbia River and samples were taken during the spawning operation at the Spring Creek National Fish Hatchery. Unfortunately it was not possible to determine the sex from the samples taken at sea therefore these samples are a composite of male and female. Table 13 shows the average blood values from the fish taken at sea, in brackish water and at time of maturity. There appeared to be an appreciable change in the hemoglobin content between the time these fish entered the river and at the time of sexual maturity.

Migrating spring chinook

The blood from adult spring chinook salmon was studied to determine morphological changes during maturity. Sixty fish were taken from the Columbia River at Bonneville Dam and transported to holding ponds at the Willard National Fish Hatchery. Twenty-five fish were killed and blood samples taken in May. Eleven of the twenty-five were females. Due to losses there were only eight fish remaining for the final sample in September, four of each sex. Of twenty samples taken in August, 12 were female.

The first blood samples taken in May displayed normal characteristics. The cells were of uniform size with good coloration and continuity. In August a greater variation between samples appeared. Moderate to severe anisocytosis was present and there was an increase in senile cells. A deficiency in polychromatophilic cells was evident in the majority of the samples. The September samples showed further evidence of degeneration. In these samples some hypertrophy appeared and a foamy or toxic appearance to the cytoplasm and karyoplasm was evident. The chromatin was dearranged and migrated to the periphery of the nucleus. It became very difficult to produce a satisfactory stain due to the sensitivity of the cells. Variations between the correlation of the cell count and the hematocrit were noticeable. This was due to change in the cell shape and the breaking down of senile cells during centrifugation (see fig. 23).

Blood regeneration studies

Trout weighing approximately 250 grams each were selected for repetitive

Table 13:--Hematology changes in salmon

Adult Fall Chinook

Combined results of male and female

	Erythrocytes	Hematocrit	Hemoglobin	Cell Size
At sea	1,190,000	52.30%	12.25 gms	--
Brackish water	1,410,000	52.80%	10.54 gms	15.5 x 10.6 microns
Sexually mature	1,295,000	48.58%	15.03 gms	14.5 x 10.0 microns

Brackish Water Adult Fall Chinook

Male	1,470,000	59.80%	10.9 gms	15.2 x 10.8 microns
Female	1,526,000	54.78%	11.74 gms	15.8 x 10.5 microns

Sexually Mature Adult Fall Chinook

Male	1,243,000	54.04%	15.3 gms	15 x 10 microns
Female	1,346,000	43.11%	14.76 gms	14.4 x 10.1 microns

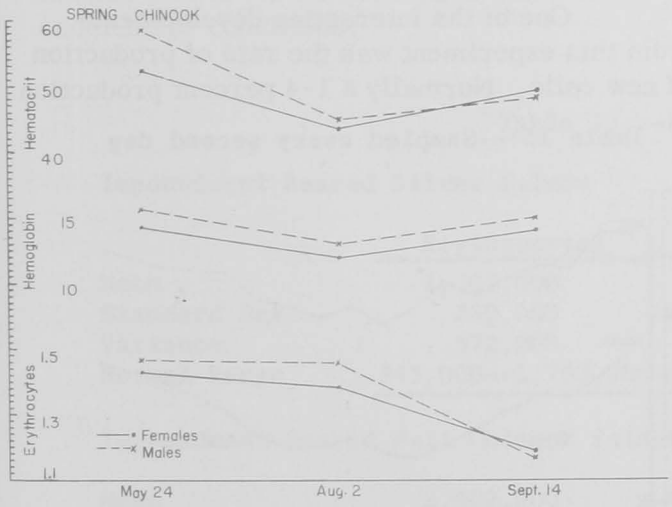


Figure 23:--Blood values for spring chinook salmon.

bleeding to measure maximum blood loss without the appearance of an obvious blood dyscrasia. During the span of this experiment the fish were bled 0.1 of its total calculated blood volume on each bleeding day. Three groups of five each were used with one fish in each group to act as a control. One group was bled every day, one group every second day, and one group every third day. The fish in each group were bled in the following manner. They were first placed in a solution of 1:15,000 MS(222) until anesthetized, then placed in a V-board and blood was drawn directly from the heart using a 5/8"-25 gage hypodermic needle on a tuberculin syringe. The control for each group was anesthetized, the needle placed in the heart and removed without drawing blood in a sham operation. It was noted at the end of the first experiment that the control fish had lost 43.4 percent RBC's, 53.3 percent hematocrit and 38.6 percent hemoglobin during the course of nine days. The fish which were samples each of the nine days had the following losses: 72.9 percent RBC's, 74.5 percent

hematocrit and 68.4 percent hemoglobin. Due to the heavy loss of blood in the control it was necessary to disregard the first experiment because of internal hemorrhage.

To prevent excessive bleeding after the removal of the hypodermic needle, injections of Vitamin K were given at the rate of 0.25 mg. per day per 250 gm fish. This was administered two days prior to the first bleeding and continued every day until the end of each experiment. Two controls were used in the second experiment, one was only anesthetized, given the Vitamin K and released. The other was subjected to the heart puncture (no blood drawn) and released. At the end of 10 days the controls were as follows: The control, which was only anesthetized displayed no appreciate drop; the second control, which received the heart puncture each day, displayed a 2.4 percent drop in total cells. The hemoglobin and hematocrit were within the same range. Therefore, it was suggested that the use of Vitamin K would minimize excessive blood loss after penetrating the heart with the hypodermic needle.

With the aid of Vitamin K to prevent excessive bleeding the experiment was set up as follows: Six fish per group with 3 groups, 2 control fish per group. One group was sampled every day for 10 days, the second group sampled every 2 days over a period of 20 days, and the third group every 3 days over a period of 27 days. In group number one, table 14, the erythrocytes show a steady decrease up through the 10th sample. The 11th sample shows a slight increase.

The hematocrit values in table 14 showed a slight increase on the 3rd sample. This increase was due to a macrocytic condition of the cells. There also appeared a severe dip on the 8th sample where a pronounced microcytic condition was evident. The hemoglobin tapered off quite gradually during the period of sampling. In the group sampled every second day, table 15, a gradual fall appeared through the 8th sample for RBC and hematocrit. At this point moderate gain was shown. The hemoglobin showed a rather uniform drop with a rise on the 5th sample and then a steady drop until the 10th sample at which time there was a gradual rise to the 13th sample. The samples taken every third day display an erratic graph. A rapid drop was followed by a modified leveling off in the 5th samples to the 9th. The group which was sampled each day showed a rather slow regeneration. Ten weeks were necessary to bring these fish back to an equivalent level of the initial sample.

Tables 15 and 16 which had one and two days between samples during the experiment showed a rapid recovery requiring only 6 weeks to regain approximately the normal values.

There was no measured changes in the sodium and potassium levels of the serum of these fish. Morphologically the blood cells developed macro and micro conditions with severe losses in hemoglobin.

One of the interesting developments from this experiment was the rate of production of new cells. Normally a 1-4 percent production

Table 14:--Daily samples

Table 15:--Sampled every second day

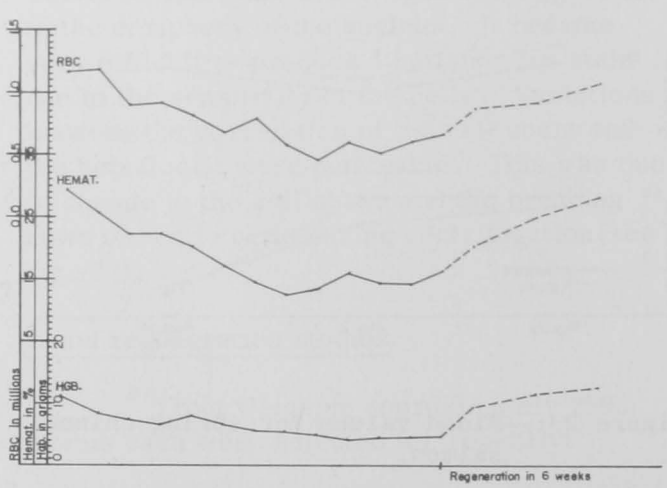
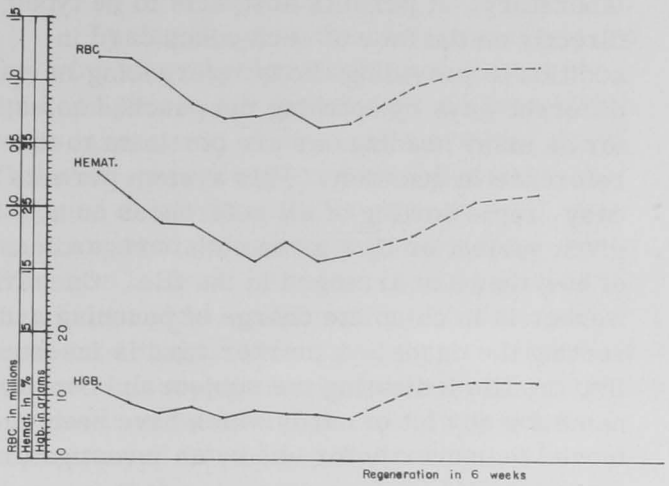


Table 16:-- Sampled every third day



of polychromatophilic cells was observed but under constant daily bleeding these fish would produce up to 10 percent and occasionally one would go over this amount. It seemed the hemopoietic system was limited to replace an immediate loss of cells. During the period of regeneration (blood drawn at weekly intervals) a build-up of 20 to 60 percent polychromatophilic cells was found.

Natural reared salmon

For the past two years a program on hematology of "natural" reared chinook and silver salmon has been conducted describing erythrocyte counts, hematocrits and hemoglobin of these fish reared in impoundments. The compiled figures in table 17 may serve as normal values for fish reared in these areas under these conditions.

White spot disease

Last year an investigation was started to determine if environmental water quality was a vector in the occurrence of "white spot" disease which has caused severe chinook salmon fry mortalities in the Columbia River hatcheries. Complete analyses were made on numerous water samples from three hatcheries in the area during the year. No major changes in water chemistry were found but occasionally significant amounts of both copper and zinc were noted. Since none of the hatcheries in the area had appreciable amounts of white spot it was concluded that if transitory water chemistry changes were responsible for past occurrences of the condition then either (a) like changes did not occur this year, (b) changes did not occur at a critical time in embryonic development, or (c) changes were not of sufficient intensity or duration to be critical.

A serious outbreak of white spot was reported at the Coleman National Fish Hatchery following the adoption of a water recirculation system. While it was not possible to examine the system or run water analyses during the height of the trouble, water analyses were taken from a modified and abbreviated recirculation system set up a month later when advice of the condition was received. In a single recirculation of water an increase of zinc and copper concentration of 0.02 and 0.006 ppm respectively was found.

Table 17:--Hematology values for salmon

Impoundment Reared Silver Salmon

	Erythrocytes	Hematocrits	Hemoglobins
Mean	1,303,000	38.89%	10.4 gms
Standard Dev.	230,000	5.4%	2.04 gms
Variance	572,000	29.52%	4.21 gms
Normal Range	843,000--1,763,000	28.09%--49.69%	6.32 gms--14.48 gms

Impoundment Reared Fall Chinook Salmon

Mean	1,402,000	43.55%	11.53 gms
Standard Dev.	210,000	4.75%	1.32 gms
Variance	452,000	22.99%	1.79 gms
Normal Range	982,000--1,822,000	34.05%--53.05%	8.89 gms--14.17 gms

An experiment has been initiated at Coleman this year. In brief, the expdrimental design is as follows: (1) Set up independent water recirculation systems in two troughs to recirculate 7-1/2 gallons of water per minute, introducing 2-1/2 gallons per minute of fresh water. (2) Maintain one system free of galvanized or brass parts while the other has the usual galvanized dam boards and pipe and brass valves. (3) Place one basket or stack of eggs in each trough on six successive weekly egg takes and then one stack of eggs eyed in the hatchery. (4) Take weekly samples of water, eggs and/or fry for zinc and copper analysis. (5) Follow mortality of individual experimental lots and mortality of the main egg take in the hatchery from which the lots were taken during incubation and for 6 weeks thereafter. The experiment is still in progress, no conclusive evidence is available.

Oxidative rancidity of fish diets

One phase of the quality control program for the Branch of Fish Hatcheries has been a continuing check on degree of oxidative rancidity in Oregon Moist Pellets. Samples from five nearby hatcheries have been analyzed by the T.B.A. method developed by Sinnhuber et al at Oregon State.

A summary by hatcheries of samples tested is as follows (at least duplicate checks were run on all samples):

Eagle Creek National Fish Hatchery	38
Little White National Fish Hatchery	14
Spring Creek National Fish Hatchery	6
Carson National Fish Hatchery	16
Willard National Fish Hatchery	5
	79

Also 4 samples of diets were assayed for Washington State Department of Fisheries.

GENERAL

Punch card bibliography

Our bibliographic filing system was mostly converted to a 5" x 8" punch card filing system during the year. This is a "master" system available to each investigator in the laboratory. It permits abstracts to be typed directly on the face of each punch card in addition to providing cross referencing in many different ways by notching the punched holes for as many headings as are pertinent to the reference in question. This system permits easy, rapid sorting of all references on a given subject or by a given author regardless of how they are arranged in the file. One office worker is in complete charge of punching and sorting the cards. A marker card is inserted into the file indicating the subject and borrower's name for any lot of cards which have been removed temporarily for use by an investigator.

Micro-film reader

A newly purchased microfilm reader-printer, Thermo-fax, permits easy reading of either 16mm or 35mm microfilm. This equipment also prints in 3 to 5 seconds, clear electrophoto copies of any page of microfilmed reference material, charts, graphs or even photomicrographs providing they are sharp, clear negatives with good contrast. This equipment extends our library facilities to any library which provides microfilm service and obviates much of the need to travel long distances in order to conduct extensive literature research and review.

Special project

Assistance was given to the Corps of Engineers, Walla Walla District, to evaluate the types of injuries sustained by fish passing through turbines. This is a fish passage program under study by the Corps of Engineers to determine what modification can be undertaken in turbine design to facilitate the greatest passage of fish downstream without mortalities. This past year a total of 5 days in January and 10 days in October was spent in evaluating the various types of injuries sustained by fish passing through the turbines at Shasta Dam. Some 3,000 fish were examined both externally and internally during the course of these experiments.

Alterations

During the year the upstairs area in the new wing was completed with installation of a lipid and physiology laboratory, a chromatography laboratory, offices and a seminar area. The multi-purpose physiology laboratory was designed to handle volatile solvents with large scale air evacuation equipment installed. This area was isolated from the remaining laboratory with independent equipment designed to minimize flash-fire danger and solvent vapor accumulation. Intricate glassware for large-scale concentration of volatile solvents and for chromatographic separation of fat soluble components was manufactured at the glass shop of the University of California, at Berkeley. This equipment was transported carefully to the new location and has been installed. The laboratory is designed to handle large volumes of various volatile solvents efficiently and with minimum of danger to facilities, equipment and personnel. With these facilities, fat and other lipid-like material can be extracted and concentrated in sufficient quantities to use in biological assay with adequate numbers of fish for sufficient growing periods to elucidate the effect of these components upon growth and metabolism.

A complete fire detection system has been installed in the laboratory with heat sensitive activator disks mounted in each room in the station and with a master locator control panel installed in the main entrance to the laboratory.

Three Navy excess house trailers were obtained and installed in the newly developed trailer park lot. Roadways and parking lots were black-topped giving a finished appearance to the station.

At Hagerman a new metabolism laboratory building was constructed to house 24 six-foot experimental tanks and with sufficient additional area for diet preparation and a small experimental animal room. With these expanded facilities winter feeding experiments can be continued for small groups of larger fish in the hepatoma induction studies conducted at this station.

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Dr. John E. Halver, Chief
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Dr. Bradford C. Croston, Chemist
Mr. Arthur N. Woodall, Chemist
Mr. Ernest F. Hesser, Fishery Biologist
Mr. Warren E. Shanks, Chemist
Dr. Laurence M. Ashley, Fishery Biologist
Mr. Robert R. Smith, Animal Husbandman
Mr. George D. Gahimer, Fishery Technician
Mr. George D. Huestis, Administrative Assistant
Mr. Pete E. Benville, Chemist
Mr. Clarence L. Johnson, Physiologist
Mr. David F. Nash, Chemist
Mr. Charlie E. Smith, Fishery Biologist
Mrs. Myrna Morones, Clerk-Typist
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Mr. Arthur C. Engel, Laborer
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Mr. Bill P. Carter, Fishery Aid
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Mrs. Bonnie F. Ternahan, Fishery Aid
Mr. Gordon C. Baker, Maintenanceman

CALIFORNIA-NEVADA SPORT FISHERY INVESTIGATIONS

Reno, Nevada
Reed S. Nielson, Chief

Plans for a 44-foot laboratory extension and two additional residences at Convict Creek Experiment Station have been approved, and bids for construction will go out early in 1963.

Comparative tests of the fitness of hatchery-reared trout for mountain stream survival continue to require almost all of the available controlled water, although additional projects, such as the year-round habitat-evaluation and food-utilization study now in progress, are included when practicable. This year's experimentally stocked trout were used primarily as a supply of environment-altered animals in an exploratory study of blood-chemistry changes under various types of stress.

A tour of California high-production trout hatcheries was completed, and a file of comparative information on water, facilities, procedures, and fish-rearing problems is on hand to aid in the design and interpretation of post-planting experiments with trout.

A study of the natural feeding of hatchery-reared trout in relation to the available food supply is continuing, with indications to date that populations of food organisms are not noticeably affected by stocked trout populations of varying density.

Shallow-water periphyton production in relation to solar illumination was studied in two divergent lake types. Production in a small, turbid crater lake was unrelated to light, indicating strongly heterotrophic activity. Autumn littoral-zone production in a broad, clear lake was largely autotrophic, with large horizontal variations resulting mainly from illumination differences.

Sampling of downstream fluctuations in particulate organic matter over an 8-mile course on Convict Creek established lake plankton and streamside vegetation as primary contributors, with variations in source and quantity dependent upon stream character and proximity to lake outlets.

Development of limnological methods and apparatus included: (a) successful functional tests of new cylindrical periphyton collectors, (b) in situ culturing of phytoplankton, (c) trials of various techniques to prevent in-storage alteration of organic matter in water samples, (d) preliminary studies of lake-sediment cores, and field testing of a piston corer.

Amphipod introductions proved highly successful in Laurel Lake, with a large and spreading population in evidence this summer. Several other trout waters have been stocked for further study of the adaptability and trout-food contributions of this small crustacean, which is commonly called "freshwater shrimp".

A study of unusual longevity (12 years) in stunted brook trout occupying a rocky-basin, high-altitude lake is in progress and will involve detailed histological and physiological investigation.

SURVIVAL AND VITALITY OF HATCHERY-REARED TROUT

In cooperation with the Western Fish Nutrition Laboratory and the California Department of Fish and Game, a test program involving several hatchery groups of catchable-sized rainbow trout was initiated in May and will continue to May 1963. This year, information on trout survival was less important than information to be derived from a schedule of blood-electrolyte, muscle-tissue, and food-habits sampling; stream stocking was based on numbers of trout to be available rather than on a per-area density fornula. The lighter stocking rates were advantageous during the runoff period following the previous winter's heavy snow, although some fish were lost in the high water.

In early May, prior to the deliveries of trout to Convict Creek, samples of blood serum, muscle, and whole trout were taken from lots held without food at Mt. Shasta, San Joaquin, Fillmore, Darrah Springs, Hot Creek, Moore-house Springs, and Moccasin Creek State Fish

Hatcheries in California. Additionally, samples were taken from two trout groups cross-transferred between Darrah Springs and Mt. Shasta water, and two between Moccasin Creek and Moorehouse Springs water, to determine possible effects of such changes upon electrolyte composition and strength.

On May 15 the test lots from Darrah Springs, Hot Creek, Moorehouse Springs, and Moccasin Creek were tank-trucked to Convict Creek Station where the sampling continued at intervals through a 7-day holding period, then at longer intervals through the summer and fall after division of the lots into stream-stocked (naturally existing) and pen-held (starvation) experimental groups.

Samples were stored frozen and transferred to the Western Fish Nutrition Laboratory at Willard, Washington, where the serum and tissue analyses are in progress.

Survival and body-condition data for these trout over the period May 22 - November 1 (census date) are indicated in the table:

	Stream-held		Pen-held	
Hatchery group	survival (percent)*	condition loss (percent)	survival (percent)*	condition loss (percent)
Darrah Springs	11.2	32.1	27.0	32.7
Hot Creek	51.7	34.1	81.5	31.1
Moorehouse Springs	69.3	31.5	77.5	30.9
Moccasin Creek	41.3	25.1	24.4	33.2

* Adjusted for removal of samples

Losses of Darrah Springs trout were unusually heavy, due primarily to the effects of severe parasitism by the blood fluke Sanguinicola davisi which produced obvious weakness in many pen-held fish. Mortalities of the Moccasin Creek trout, which began at smaller size and lower body condition than the others, increased rapidly during fall months, particularly in the starved group.

Following the November census, the remnants of all stream groups were confined in a single stream section as a sample pool to facilitate the winter trout-food study and later blood work. Mortality during December has been moderate among the remaining fish except for a heavy loss of starved Moorehouse Springs and Hot Creek trout, delayed because of earlier resistance supplied in part by higher initial body condition and better health. Overwinter survival cannot be studied meaningfully this year, as periodic samples plus natural mortality will liquidate one or two of the populations before a spring census can be made. Presently active groups of starved trout (3) will be held to LD_{100-10} for each, at which times final blood and chemical samples will be taken.

BIOLOGICAL EVALUATION OF STREAM SECTIONS

A two-year study of relative densities and types of trout food organisms (obtained with the Surber square-foot sampler) will continue on a semi-monthly basis until June 1963. Information to date shows a definite order of production among the sections with the average 1962 standing crop of each section being: Section I - 114 lbs./acre; Section II - 136 lbs./acre; Section III - 100 lbs./acre; Section IV - 146 lbs./acre. The dominant organisms by weight were aquatic oligochaetes, whereas caddisfly larvae (Lepidostoma) were the most numerous. Indications are that stream-bottom food production in the experimental stream remains seasonally stable whether hatchery trout are present or not present.

Density and distribution of principal food organisms are also being related to stream velocity, volume of flow, bottom type, width, depth, shade, and water temperature.

FEEDING HABITS OF HATCHERY-REARED RAINBOW TROUT

Stomachs for food analysis are being collected from catchable-sized rainbow trout representing four State of California hatcheries. The trout were maintained in four separate

stream sections from May to November, during which time semi-monthly collections were made by fly-fishing. Samples for the remainder of the survey period (to May 1963) will be taken at monthly intervals, and food-habits data will be correlated with accumulated information on foods available in the stream.

QUALITY OF HATCHERY-REARED TROUT IN RELATION TO CULTURAL CONDITIONS AND PROCEDURES

Data on the chemical quality of 13 California hatchery water supplies were consolidated and arranged for various methods of breakdown to assist in comparisons of production, initial quality, and post-planting performance of trout. Inspections and interviews were conducted at 13 California trout hatcheries for the purpose of evaluating features and problems peculiar to individual hatchery operations (water quality, disease, feeding, use of water supplies, etc.) and their possible roles in determining trout quality. The nature of the information obtained will probably limit its analytical use to the trout-vitality experiments planned or in progress at Convict Creek.

WATER QUALITY AS A FACTOR IN TROUT REARING AND TRANSFER

A repeat of the 1961 experiment to determine the effects of rearing in two radically different hatchery waters, followed by transfer to Convict Creek, was initiated May 15 and terminated November 1. Two groups of rainbow trout from a single egg lot, reared to catchable size at Moccasin Creek Hatchery (12 ppm. TDS, 0.5 ppm. Ca)* and at Moorehouse Springs Hatchery (335 ppm. TDS, 95 ppm. Ca)* were tested for reactions to change in water by (1) transferring trout from one hatchery to the other and vice-versa, and (2) transferring from the hatcheries to holding pens at Convict Creek (77 ppm. TDS, 22 ppm. Ca)*. In terms of distress and mortality there were no indications this year that transfers among these particular waters are harmful to the trout. (In the previous test, trout from Moorehouse Springs sustained a 26 percent loss in 12 days following transfer to Convict Creek. At the time the mortality was tentatively attributed to a form of osmotic shock; however, in view of the trouble-free transfers this year it appears that other factors are involved.)

Later survival tests in the stream essentially repeated the 1961 results as to relative performance. The indicated superiority of the Moorehouse Springs trout was again considered to be influenced strongly by their larger initial size and better body condition.

HEMATOLOGY COMPARISONS

Following the close of a pen-holding experiment in November, two groups of rainbow trout from two hatcheries were sampled for blood condition (hemoglobin, hematocrit, RBC), then released in an unused stream section. Later in the winter they will be sampled again for evidence of improvement. The initial blood samples will give the condition at the end of a 12-week starvation period; later samples are expected to provide indications of any benefits available from limited winter feeding in the stream.

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An early season ice cover on Laurel Lake prevented the study of fall periphyton production there (an expanded experiment based on a study of the previous year which was formally presented at the XV International Limnological Congress in August). Alternatively, shallow-water periphyton production was studied in two divergent lake types: small, turbid Inyo Crater Lake Jean and broad, clear Convict Lake.

Sets were positioned at 0.5, 1.0, and 1.5 meters in depth at each of four stations in the one-acre crater lake. The stations were located marginally at the primary compass points in order to obtain maximal differences in illumination conditions. Periphyton collecting cylinders, removed after 33 days, appeared to have moderate algal growth but analysis showed that most of the material was inorganic. Average

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organic accumulation was about 0.9 mg. per square decimeter of collector surface area, and little variation was found with either depth or location, except that the west side station yielded only 0.6 mg/dm^2 at all depths. The low production and its lack of relationship to light suggest that heterotrophic organisms were responsible--probably bacterial colonizations which attracted and retained large amounts of inorganic particles.

Convict Lake periphyton growth was assessed during November in four littoral areas representing, as in the above lake, places differing strongly in the amount of light radiation received. In each area, three stations were arranged as a short transect perpendicular to the shoreline at water depths of 2, 6, and 10 meters. Periphyton collectors were set at odd-meter depth intervals totalling 9 sets per transect and 36 sets overall.

The collectors were removed after 34 days during which time unprecedented growth occurred, primarily due to the predominance of a single species of the filamentous diatom, Tabellaria. Lake-average production was highest at a depth of one meter, 40 mg per square decimeter of collector surface area, and declined steadily to a minimum of 10.5 mg/dm^2 at 9 meters (an average decrease of 3.7 mg/dm^2 per meter in depth). Production differed in each area: highest occurred in the North and East littoral areas where sky exposure is the greatest; the South area, shaded by the lower shoulders of 12,268-foot Mt. Morrison, produced the least and had the only logarithmic decrease with depth (simple light dependency relationship); surface light inhibition was limited to the North and West areas which receive the largest proportion of high-angle direct sunlight. The extreme production values observed were 58.5 mg/dm^2 at a depth of 1 meter in the East area and 2.5 mg/dm^2 at a depth of 9 meters in the South. It is concluded from these data that the fall autotrophic production in the littoral zone of Convict Lake may vary up to 10-fold at a given stratum in different parts of the lake and that these variations are associated primarily with changes in the light environment.

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A long-term sampling of the microscopic particles in waters of this area was initiated in order to determine the characteristic lake nannoplankton and the temporal changes of particulate organic matter in Convict Creek. In addition, a pilot study was made of the downstream changes in the particulate organic content of Convict Creek. Five samples, taken the same day at different stations along an 8-mile route, yielded the information shown at the top of the next page.

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New cylindrical periphyton collectors functioned satisfactorily in recent periphyton studies. The 2 x 5/8-inch glass cylinders, substituted for the microscope slides previously employed, accumulated a generally homogeneous coating and allowed up to 23 organic analyses to be made simultaneously--thus

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Sampling Station	Elevation in feet	Stream miles below previous sample	Particulate organic content*	
			General character	Quantity, gcal/liter
Outlet of Cloverleaf Lake	10,300	0	phyto-plankton	4.1
Outlet of Lake Genevieve	9,900	1.5	phyto-plankton	2.2
Creek above Convict Lake	7,600	3.5	organic detritus	0.7
Outlet of Convict Lake	7,600	1.0	phyto-plankton	2.2
Creek at Experiment Station	7,100	2.5	organic detritus	3.3

* Ranging from colloidal material to 0.3 mm in size

obviating two disadvantages of the flat-plate collectors. The average deviation in the quantity of periphyton occurring on 30 paired sets was 21 $\pm$1.8 mg/dm^2 or $\pm$ 8.6 percent.

The culturing of native phytoplankton in situ in plastic bags in order to evaluate light and temperature influences has not yet proved satisfactory. An unexpectedly long lag phase of algal growth (4 weeks) resulted in termination of the pre-scheduled 6-week experiment before maximum culture development was attained. A second test, conducted in a small crater lake, produced no positive results--possibly because of several peculiar environmental factors.

Current investigations concerning the preservation of organic matter in water samples during storage indicate that:

1. The total organic content of unfixed lake water samples kept "on the shelf" may increase or decrease measurably within a month. While the decrease is due largely to autolytic decomposition, the increase appears to be associated with light-induced growth occurring inside clear (glass or plastic) bottles.

2. Ten to twenty mg/l copper as $CuSO_4$ seem to inhibit both growth and decompositional changes, but analytical results so far have been inconclusive.

3. Brown-glass bottles and refrigeration combined gave more positive results in minimizing changes in organic content of the water during a month's storage.

Advantage was taken of the early ice cover on higher lakes, lack of snow, and warm days to construct and field-test a core sampler (fig. 1). The piston corer was fashioned from sections of lightweight alloy life raft oars and was tested on lakes which are usually inaccessible by late fall. Lake sediment cores up to 50 cm long can be taken with the present device in water up to 6 meters in depth (fig. 2). The preliminary corings suggest that lake sediments of this area might provide a key to the post-glacial changes in general productivity and environmental conditions as well as an index of the relative productivity of individual waters.

ENVIRONMENTAL MANIPULATION; STOCKING OF BENEFICIAL TROUT-FOOD ORGANISMS

The objective of this project is to measure the success of transplanting floral and faunal elements for the improvement of alpine aquatic food chains which ultimately benefit trout populations. At present the program is limited to experimental introductions of locally available crustaceans (principally the freshwater amphipod Gammarus lacustris) and to pilot observations on environmental tolerances of some aquatic weeds. This year,

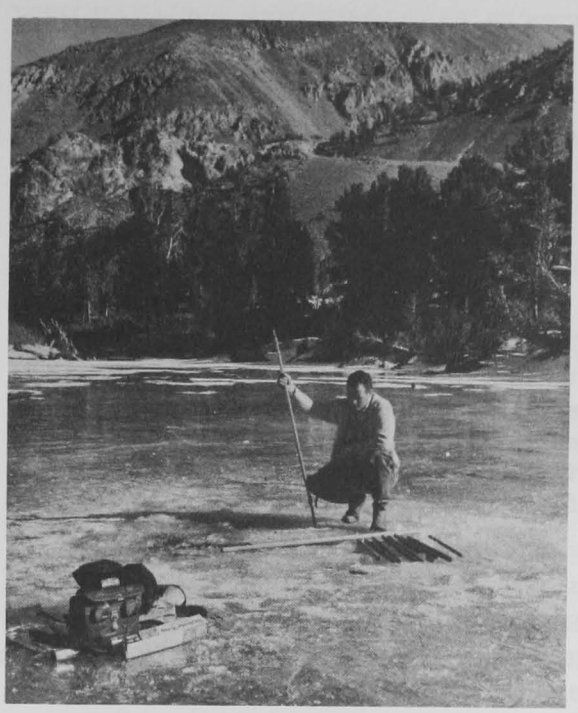

Figure 1:--Core sampling the bottom sediments of a small, ice-covered Sierra Nevada lake.

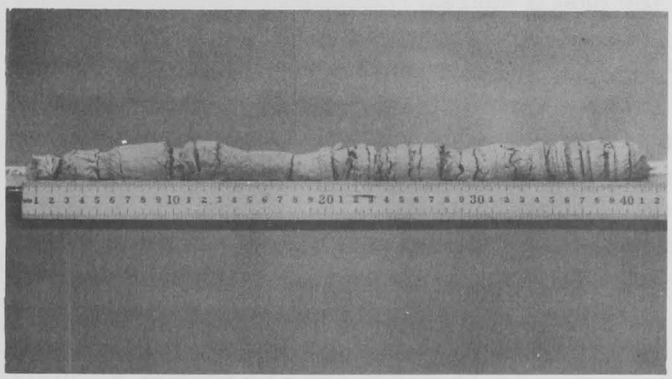

Figure 2:--Dried sediment core from a 1500-year-old crater lake. Degree of shrinkage is proportional to the organic content.

following installation of siphon collecting devices in a heavily populated spring, approximately 150,000 Gammarus were distributed in six selected waters which will be sampled periodically for evidence of reproduction and population development. An attempt is being made to assess the range of alpine habitat conditions suitable for this versatile food organism, insofar as local variation will permit.

The 1959-60 stocking of Upper Laurel Lake (9,500 feet elevation) was shown to be highly successful by this summer's dredge sampling, which yielded as many as 106 amphipods per 1/4-square-foot on the most favorable lake-bottom areas. Previous samples (1961) had shown a spread from the stocking point to only about 1/3 of the lake's bottom area; this year, all parts of the bottom were occupied to some extent. Observations will be continued to determine if this population will extend downstream to a second lake.

Samples of trout were removed from two higher lakes (10,000 feet) for scale reading to fix growth rate prior to the introduction of Gammarus, and post-introduction measurements will be made if warranted. Absence of early snow this year has made continued collection of amphipods feasible despite a few freeze-ups of equipment, and two study areas were stocked in December. One of these is a warm, spring-fed stream tributary to Crowley Lake (a productive reservoir in which the organism does not occur), and the absence of heavy ice in this area may afford an opportunity for intensive seeding this winter with follow-up measurements of spread into the lake.

BROOK TROUT OF BUNNY LAKE

Surviving members of a population of brook trout stocked experimentally in 1951 reached the record age of 12 years in 1962. Stunted (present average length 7.6 inches), and showing superficial signs of old age, these trout have never reproduced in all the years they have been observed. The virtual absence of growth in an overpopulated and marginal 11,000-foot environment is easily understood, but the attainment of an age more than twice the expected life-span of the species under these conditions invites explanation (fig. 3). A study to relate the unusual longevity to physiological economies peculiar to the habitat type was activated this year, and will probably require most of 1963 for completion of a first report.

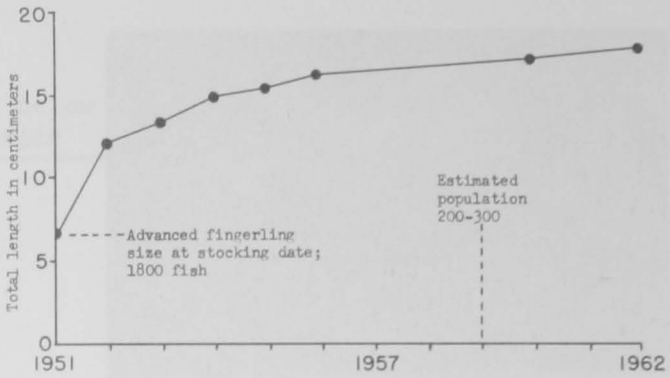

Figure 3:--Lifetime growth curve for brook trout in Bunny Lake. (Note that population reduction in later years has not influenced growth rate upward)

Field work in 1962 included the collection and preliminary preparation of trout tissues for histological study. This material is now being processed and studied by the collaborating histologist at the Western Fish Nutrition Laboratory. Organs and tissues of younger brook trout will be examined for comparative evaluation of senility factors in the aged fish.

Several specimens were successfully hand-carried in polyethylene bags from Bunny Lake to the Convict Creek Laboratory for various live-testing purposes. The single survivor of the transfer is being maintained in a hatchery trough with daily feedings of natural food organisms; attempts to substitute hatchery feeds have failed. This individual (affectionately known as Djaughe) has grown 1.5 cm in length and 13 gm in weight in three months, slightly more than the average growth in the lake (1.3 cm) over the past 5 years.

STAFF

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

Construction of 45 ponds ranging from 0.1 to 1.0 acre in size was completed early in the year. Formal acceptance of the ponds was made on April 27.

Following severe problems involving numerous breaks in the water mains, the ponds were filled and stocking of the ponds began on June 4.

The poor quality of the available water led to the construction of an aeration-filtration plant to remove excessive amounts of iron. This plant was put into operation on August 20.

Ceremonies for the official dedication of the station were performed on October 21.

Fish barriers were constructed of a saran-type material which successfully prevented the entry of wild fish through water supply systems which utilize surface water.

An organophosphate insecticide, Guthion, was found to be effective in the removal of other species of fish from waters managed for catfishes.

A thorough investigation was made of the differential toxicity of oil formulations of benzene hexachloride as opposed to wettable powder preparations.

A survey was completed of the water qualities found in the Grand Prairie region surrounding the station. Major drainages which may furnish water for use at the station were sampled.

DEDICATION

The Fish Farming Experimental Station was officially dedicated on October 21, 1962. Despite inclement weather, approximately 250 persons gathered to hear addresses by Senator J. William Fulbright, Commissioner C. F. Pautzke, Regional Director, Walter Gresh, and from representatives of the Bureau of Commercial Fisheries, Soil Conservation Service, University of Arkansas, Arkansas Game and Fish Commission, and the Stuttgart Chamber of Commerce. An open house with numerous displays showing the nature of research carried on at the station followed the ceremony.

FISH CULTURAL INVESTIGATIONS

Fingerling fish for the initial stocking of the ponds at the station were received through the cooperation of the Branch of Fish Hatcheries from their hatcheries in Arkansas, Alabama, Georgia, and South Carolina. Brood stock for experimental purposes were collected from local waters, State hatcheries, and from private individuals.

Attempts to perform some cross-breeding between species of buffalo met with no success since it was impossible to keep ferric hydroxide from accumulating on the eggs. Egg trays and hatching jars became so filled with the rusty precipitates that all units had to be discarded.

Channel catfish and flathead catfish were successfully spawned in aquaria and holding tanks using water held for one week in a pond and then pumped into the auxiliary building. Water quality again caused considerable difficulty and several lots of fry were lost due to excessive accumulations of the ferric hydroxide despite daily flushing and cleaning of the troughs.

Three additional species of fishes have been secured for experimental use at the station. These include the white catfish, Ictalurus catus, the speckled bullhead, Ictalurus nebulosus, and the Tilapia mossambica.

DISEASE INVESTIGATIONS

A disease condition known locally as "winter-fungus" was isolated, cultured, and identified. The condition is actually a complex of diseases, usually involving a bacterium and a fungus. Most commonly, Saprolegnia sp. fungi and Aeromonas liquefaciens are the etiological agents although columnaris infections

are common. Susceptibility tests were run and several promising drugs were discovered. Chloramphenicol was most effective but is too expensive for use in treating large bodies of water. Although Gentian Violet, and Malachite Green did control the fungus, they did not control the bacterial disease. Furazolidone showed some promise but did not control the fungus. Combinations of the latter compound with Malachite Green or Gentian Violet did not yield consistent results.

Field trials were conducted using four new systemic insecticides for the evaluation of their effectiveness in controlling anchor parasites. These materials were added to ponds after preliminary testing in the laboratory. Coral and Ruelene did not control the parasites. Dylox and Korlan gave positive results and will be tested further during the next growing season.

Extensive laboratory tests were completed to determine the causes for the increased toxicity of oil formulations of benzene hexachloride over dust preparations of the same insecticide after a fish farmer reported a loss of 185 acres of minnows due to the use of an oil preparation. Tests were run of the active ingredient, the solvent systems, and on the various emulsifiers and detergents used in formulating the commercial preparations. Results indicate that there is no difference in the toxicity of the active ingredient used in the two preparations and that the various ingredients are not toxic to fish at the levels commonly applied. However, the addition of a hydrocarbon increases the toxicity of the insecticide to approximately 20 times that of the dust formulations. Further studies are planned in an effort to establish the cause for this condition.

Additional cases of algal toxicosis were discovered. Evidence now on hand indicates that extensive fish losses may occur in ponds having extensive blooms of blue-green algae although oxygen supplies are adequate. Algae generally involved are Microcystis and Anabaena.

Approximately 7 percent of the catfish produced in the experimental ponds show serious deformities of the spine. Serious lordosis and scoliosis is evident in most of the affected fish. The condition is sufficiently advanced in certain individuals that circulation to the caudal peduncle is impaired and the resulting atrophy has resulted in complete loss of the caudal fin. Causes for this condition have not been identified.

Feeding experiments, initiated eight months ago, failed to produce any experimental infections of myxosporidia in golden shiners. Exposed fish were sectioned and examined with negative results. Surviving fish show no symptoms of developing the disease.

Diagnostic services were provided to inquiring fish farmers throughout the year.

PHYSIOLOGICAL INVESTIGATIONS

Sixteen ponds were stocked with fingerling channel catfish in June and fed various formulations using local agricultural by-products as substitutes for the more commonly used ingredients in an effort to find a low cost feed suitable for fish production. Growth rates, condition factors, hematocrits, and other pertinent data were collected in November and the fish were returned to the ponds for an additional year of growth.

A study has been made of the various types of feeds which have been utilized in the production of catfish. Various agricultural by-products were considered and the potential market for supplemental feeds was examined. A manuscript of this report has been prepared and submitted for publication.

The handling of large fish, especially brood stock, is often difficult since it is impossible to get good holds on the fish. Such fish often are injured when attempts are made to transfer them from ponds to holding tanks. Experiments were conducted using various anesthetics in an effort to find a compound which could be injected intramuscularly, act quickly, and have short duration. Sodium pentothal, Anectine, Brevital, Tubocurarine, Sucostrin, and Kemithal were tried with negative results from all compounds.

Hematocrits taken from 350 channel catfish (10.6"-12.7") as they were checked from the ponds in November yielded an average of 33.4 (range: 30.1 - 37.8). This is considerably higher than figures from fingerling fish (6"-8") taken in January. The mean for 75 fingerlings in apparent good condition at that time was 27.0. It is therefore apparent that considerable influence may be exerted on hematocrit values by the size of the fish, the season of the year, or the conditions under which the fish are taken.

LIMNOLOGICAL INVESTIGATIONS

Water chemistry data, plankton analyses, and bottom fauna determinations were collected throughout the year according to a prescribed schedule on all station ponds. A recording thermometer was installed to follow temperature changes in a typical pond. The data collected thus far indicate a significant difference between ponds filled with ground water and those filled with surface water. Ground water generally gives a higher pH and higher carbonate and bicarbonate alkalinities. Surface water is usually low in dissolved solids, nearly neutral in pH, and contains high levels of suspended solids in the form of colloidal clay. Such turbid water tends to reduce plankton blooms and ponds filled from this supply seldom develop oxygen depletions.

Several fertilizers and combinations of fertilizers were tested in preliminary studies to determine their use in stimulating and maintaining plankton blooms. Superphosphate (46 percent P_2O_5) and nitrogen in the form of ammonium sulfate, urea, or a liquid preparation were applied to 0.1-acre ponds. Data collected from this short study indicate that amounts of both nutrients are required to produce and maintain a desirable plankton bloom. Use of only one nutrient resulted in poor plankton populations or in blooms of undesirable forms such as blue-green algae.

During the past two years, selected reservoirs in the vicinity of the station which are used in the commercial production of fish were sampled to provide background information for experimental work. These reservoirs included a variety of water sources which are more or less typical of fish farming operations. Samples were taken on a scheduled basis and analyzed at the station. Data accumulated in this study have been organized in the form of tables listing water temperatures, pH, CO_2, alkalinity, dissolved oxygen, iron, and specific conductance. Plankton analyses include determinations of species of algae and of the various invertebrates which were found in each collection. These reservoirs are being harvested and data from harvest figures will be studied in an attempt to find some degree of correlation between water quality and production.

Analyses of soil samples taken from station ponds prior to filling were completed. This information is now on file and will be used as reference data in the future.

A series of station ponds was subdivided into four plots each through the use of polyethylene sheeting to provide experimental units in which fish-rice rotations can be tested on a small scale. The first crops were produced during the growing season just completed. During the next three years, the plots will be rotated between the following alternatives: Fish, water without fish, rice, and rice with fish. During each year samples will be taken from the soil in an effort to determine just how raising fish increases rice production.

A survey was made of the water quality in the vicinity of the station at Stuttgart and the undeveloped area near Kelso. The Stuttgart area is served primarily by two major drainages, Bayou Meto and Bayou Lagrue. During periods of high water and heavy rainfall, there is little difference between the two systems. During the summer, when the flow is lowest, there is a striking difference between the two drainages. Due to its smaller water volume, Lagrue is affected to a higher degree by irrigation waters drained from rice fields. This is most apparent in higher levels of dissolved solids. Waters in Desha County (near Kelso) contain much higher levels of total iron than waters in the vicinity of the Stuttgart station.

FISH MANAGEMENT STUDIES

A saran material was tested for its effectiveness in barring the passage of fish, fry, and fish eggs. The material has proved to be most effective and several types of barriers were constructed and tested for their adaptability to the various situations provided in fish farming operations. A manuscript on the use of this material has been prepared.

A report on the current trends in fish farming was prepared based on findings in survey work, discussions with fish farmers, and through efforts in conjunction with the Bureau of Commercial Fisheries. A manuscript has been submitted for publication.

One-acre ponds were stocked in June with various combinations of fish and in varying ratios in an effort to gather preliminary data on the most desirable stocking rates and on growth rates of the stocked species. This experiment is not completed although data were collected in November when the fish were weighed, measured, and returned to the pond for another growing season.

A cooperative project was initiated with a local rice farmer in which fingerling channel catfish were stocked in a 20-acre field planted to rice. The field was kept flooded with a minimum of 6 inches of water and with approximately 2.5 feet of water in the irrigation ditches. Three thousand channel catfish fingerlings weighing approximately 0.02 pound each were stocked in the irrigation canals. After one week of confinement in the canals, the fish were provided access to the field by cutting openings through the levees. Three months after stocking, the field was drained to facilitate harvest of the rice. Water was slowly drawn off the field to prevent entrapment of the fish in the rice and to encourage the fish to return to the canals. Of the 3,000 fish originally stocked, only 315 were recovered. These fish averaged slightly more than 0.1 pound. Although this attempt to raise catfish with rice was considered a failure, the fish had no adverse affect on the rice crop since the yield exceeded 100 bushels per acre.

A series of eight 0.1-acre ponds at the station was stocked with channel catfish to determine the utilization of natural foods by this species. Certain ponds were stocked with shiners, others with fathead minnows, while still others received only fertilizer to stimulate plankton production. Analyses of the data are not complete at this time but results show that channel catfish will not feed on minnows until the catfish reach a size of eight or more inches.

Field trials based on previous laboratory studies concerning the use of Guthion as a selective fish eradicator were completed during the year. These trials indicated that catfishes are more tolerant to levels of Guthion than the other species of fish included in the experiment. All other species were quickly affected by

The toxicity of Guthion* (25% wettable powder) to five species of fish in a 48 hour period under laboratory conditions.

Species	LD/0	LD/50	LD/100
Largemouth bass (20)	.005 ppm active	.025	.050
Green sunfish (20)	.005	.025	.050
Bluegills (20)	----	----	.025
Golden shiners	.050	.10	.20
Channel catfish (20)	5.0	9.0	12.0

* Chemagro Chemical Corporation, Kansas City, Missouri.

applications of 1 ppm. A complete kill of the other species was effected in 48 hours. The compound performed effectively under various water conditions which included highly turbid water from a bayou, relatively clear water from a large lake, and in ponds filled with ground or surface water. The data indicate that applications of less than 1 ppm Guthion

Figure 1

Figure 2

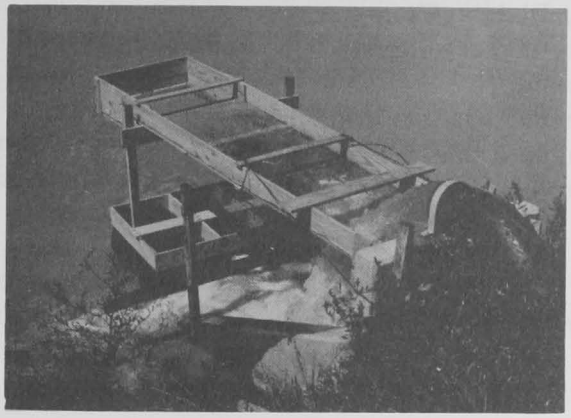

Figure 3

Figure 4

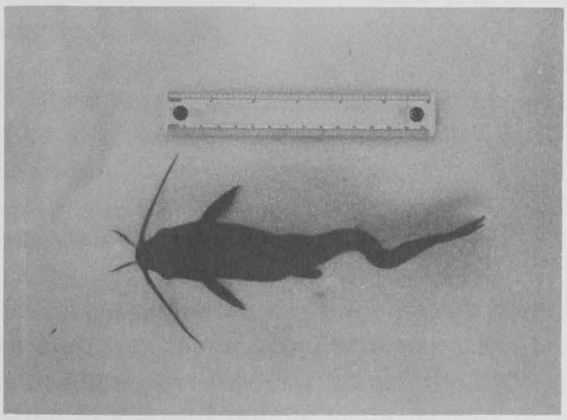

Figure 5

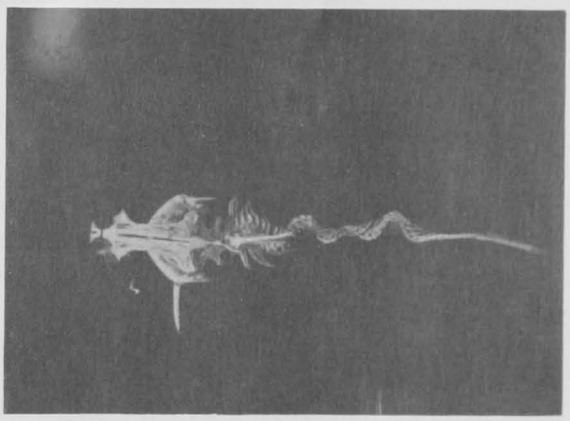

Figure 6

Figure 1:--Completed facilities at the time of dedication of the Fish Farming Experimental Station.
Figure 2:--Senator Fulbright and Commissioner Pautzke inspect exhibits during the open house following dedication.
Figure 3:--Box type fish barrier constructed of saran screen used with 1000 gpm water discharge.
Figure 4:--Sock type fish barrier constructed of saran screen used with 1800 gpm water discharge.
Figure 5:--Deformed channel catfish: Dorsal view.
Figure 6:--Deformed channel catfish: Dorsal view of skeleton.

will effectively remove green sunfish without apparent effect on catfish. In addition, significant numbers of other undesirable species can be removed with comparative safety. As such, the compound should have potential usefulness in reducing the amount of sorting required during harvests at catfish hatcheries and should help provide a way to renovate farm ponds managed for catfish production. Fish farmers might also be able to fill their reservoirs at any time of the year with surface water and subsequently remove the undesirable species without affecting the stocked catfish. A manuscript of this report is in preparation for publication.

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

The development of an adequate, reconstituted all-meal diet for fall chinook salmon fingerling appears imminent.

A more sensitive colorimetric method of oxygen measurement has been developed.

A program designed to define the fingerling characteristics necessary for maximum adult survival has been initiated.

A direct correlation between the percentage body protein and performance of the fingerling has been demonstrated.

An increase in fingerling stamina can be induced by exercise. In exercised fish, performance increases with size. In raceway-reared fish, however, performance remains static after the fish reach 5 grams in weight.

Estrone appears to be successful in accomplishing sex reversal in male fall chinook salmon fingerling.

Trematode cercariae in hatchery water supplies may be successfully controlled by electrocution.

GENERAL

The weather during the past fall was, to say the least, unusual. On October 12, winds of hurricane velocity struck the area resulting in a power outage at the station of 10 days duration. Aside from the inconvenience and time required to remove the fallen trees the station suffered no damage. In November, the largest flood since 1933 occurred. Again, the station came through unscathed. In the interim between the hurricane and the flood a minor earthquake was experienced. Aside from these occurrances, operations were normal.

The station produced 1,077,000 fall chinook fingerling weighing a total of 26,500 pounds. The adult chinook run amounted to an estimated 500 fish. Of these, 200 were diverted into the holding pond and 200,000 eggs from 50 females were taken for artificial propagation. Some of the takes were heavily affected by coagulated yolk causing high losses in the resultant fry. Three million fall chinook eggs were transferred from the Spring Creek National Fish Hatchery. These fry also showed coagulated yolk although not to the extent of the native run. All fish are now in rearing ponds and feeding well.

Personnel of the laboratory attended several meetings during the year. Burrows attended the American Fisheries Society meetings in Seattle and Jackson Hole and presented a paper on the effects of excretory products on hatchery-reared salmonids at the latter meeting. A paper was also presented at the annual meeting of the U. S. Trout Farmers Association. The entire staff hosted the Northwest Fish-Cultural Conference held at Longview on December 4 and 5. There were 187 in registered attendance. Members of the laboratory staff presented progress reports on the various phases of our investigations.

APPLIED NUTRITION STUDIES

1961 feeding trials

This project was completed with the publication of the Special Scientific Report: Fisheries No. 432 "Protein and Calorie Levels of Meat-Meal Vitamin-Supplemented Salmon Diets".

1962 feeding trials

The 1962 diet experiments conducted with fall chinook salmon were a continuation and enlargement of the 1961 trials. The purpose of the experimentation was to define optimum protein and caloric levels in a high meal diet and to determine the amount of vitamin supplementation necessary for adequate maintenance. The protein and caloric intakes were controlled by the use of water as a dilutant. In this manner the protein and caloric levels in the diets as fed could be maintained or varied in any desired amount merely by varying the amount of water or lipids added to the standard

mixtures. The partially reconstituted diets were bound by the addition of salt and CMC and ricer fed. The amount fed was allocated to the individual lots on the basis of body weight and water temperature.

After 24 weeks of feeding at a constant water temperature of 53° F. the results were as follows:

1. All diet fish were demonstrating symptoms associated with vitamin deficiency at the conclusion of the experiment despite high levels of meat and/or purified vitamin supplementation in some rations. The degree to which the symptoms had progressed was correlated with the protein and caloric levels fed.

2. Protein utilization as measured by protein fed and protein deposited was most efficient at the 25 percent level of protein intake when compared with 20 percent and 27.5 percent levels.

3. At the 25 percent protein level an increase in the caloric intake from 1,650 to 2,350 by the addition of peanut oil significantly increased protein utilization and decreased symptoms of the hypervitaminosis. A sparing action on the protein and vitamin requirements of the animal is indicated.

4. Body composition changed during the last 12 weeks of the experiment. While the protein remained essentially the same, the percentage of fat decreased. A proportionately higher energy utilization in the larger fish is indicated.

5. Fish from diets which showed the most advanced vitamin deficiencies had lower stamina than those in which the syndromes were marginal.

6. The composite meal diet proved as good or better than its meat-supplemented counterparts. Protein utilization, survival, performance, and general condition throughout the 24-week experimental period were excellent. This meal combination with slight additional vitamin supplementation appears to have excellent potentialities as a production diet for chinook salmon.

The final report on the 1962 diet trials, scheduled for completion in March 1963, will conclude this work unit.

EVALUATION OF ENVIRONMENTAL FACTORS LIMITING PRODUCTION IN REARING PONDS

Biological demand of fingerling for oxygen

Preliminary exploration of this problem has resulted in the development of a more refined method of oxygen measurement. The report "A Sensitive Photometric Procedure for Dissolved Oxygen with Potential Field Application" has been accepted for publication in The Progressive Fish-Culturist. It has been demonstrated that within an age group the larger fish have a higher oxygen requirement probably associated with a higher metabolic rate and consequent faster growth rate.

Throughout a 24-hour cycle the oxygen demand of chinook fingerling varies. A daily rhythm in the demand is indicated. This rhythm is not associated with feeding, temperature, or abnormal activity although all these factors do affect the oxygen requirement.

Determination of limitations imposed by excretory products on carrying capacity of rearing ponds

This project is complete. Results of the investigations were presented at the annual meeting of the American Fisheries Society and a final report has been prepared for publication. Briefly, ammonia and particularly un-ionized ammonia was found to be the catabolic product limiting production in rearing ponds.

MEASUREMENT OF DIFFERENCES IN CHARACTERISTICS OF FINGERLING SALMON

Measurement of physiological and chemical differences

This program includes investigations to determine some blood plasma constituents, body composition, gross hematology, and gross pathology of fingerling salmon at time of liberation. The objective is to determine if measurable differences exist in these characteristics and if these differences affect stamina and ultimate survival.

More than 30 pooled samples of blood plasma from stamina-tested fall chinook salmon fingerling have been analyzed for total protein, albumin, glucose, cholesterol, creatinine, uric acid, urea, ammonia, phosphorus, calcium, and chloride. Measurable differences between plasma samples were demonstrated for total protein, glucose, and cholesterol. The significance of these differences is yet to be determined. Difficulties have been encountered in the ultramicro techniques employed in some of the measurements and refinements in these techniques are indicated. In future analyses albumin, ammonia, creatinine, urea, uric acid, calcium, and chloride will not be included either because no measurable differences existed or the techniques were unreliable. This deletion does not preclude the possibility of reinstatement of some of these measurements when techniques are stabilized, or the introduction of new measurements as procedures are developed.

The alteration in body composition due to the diets fed in different hatchery regimens is easily demonstrated. Tests to date indicate a direct correlation between the percentage body protein and performance. Conversely, an inverse correlation exists between percentage of fat and performance. At the present time it appears that diet formulation should be directed toward producing fish of high protein and medium to low fat composition if high performance is desired.

Plans are in progress to expand the hematological evaluations to include the number and size relationships of the erythrocytes in the samples. Such counts will be made by means of an electronic counter which is fast enough to make the procedure feasible. In hatchery samples tested this season, none was found with an average hematocrit below 30 percent, the critical elvel found to affect performance. Abnormally high hematocrits of 45 percent were found to be associated with hyperplasia of the gill epithelium.

In the gross pathology studies, the most obvious relationship is between stamina and gill condition. Any protozoan or bacterial disease or environmental condition which has resulted in extensive proliferation of the gill epithelium is reflected in reduced stamina when the animal is stressed.

Evaluation of a stamina tunnel as a method of measuring fingerling differences

Tests to measure and define the effects of controllable variables on the performance of fingerling salmon have continued in 1962. The normal performance curve of raceway-reared fall chinook fingerling between the average weights of 1.0 to 3.7 grams has been ascertained. The greatest acceleration in performance was between the 2-gram and 3-gram levels. It is during this period when the swimming ability of the fish is showing the greatest increase. Above 3 grams in weight the rate of increase in the performance index declines. In raceway ponds, no increase in stamina is indicated as the fish increase in size from 5 grams to 20 grams in average weight with the index stabilizing at 28.5 ± 1.5. In the recirculating raceway a gradual increase in the performance index up to 40 is indicated over the same size range. We feel that the capabilities of fish are not developed in the raceway due to insufficient exercise.

DEFINITION OF FINGERLING CHARACTERISTICS NECESSARY FOR MAXIMUM ADULT SURVIVAL

Determination of the effect of stamina differences in fingerling on adult survival

The first phase of this experiment was completed with the release of two lots of marked fall chinook fingerling on September 18 and 19. One lot of 199,000 marked by the excision of the right pectoral fin was raceway-reared and had a performance index of 30. The second lot of 182,000 fish marked by excision of the left pectoral was reared in the rectangular-recirculating ponds and had a performance index of 40. These were significant differences in performance imposed by subjecting the fish to higher current velocities in the rectangular ponds while maintaining other factors comparable. The adult returns in 1965 will indicate if stamina is a factor in survival.

SEX CONTROL IN SALMON

Development of method for sex control

The exposure of fall chinook salmon eggs to suspensions of estrone during the period of water-hardening appears to have definite possibilities as a means of inducing sex-reversal in males.

Development of a method for inducing sex reversal has been fraught with frustrations. In the first experiment poor water circulation in the experimental lots caused erratic mortalities making it difficult to determine if sex reversal occurred or if a selective mortality was present. Our analysis indicated a definite sex reversal. A second experiment using rainbow trout eggs resulted in failure. In this experiment the technique of preparing the estrone dilutions was not a duplicate of the first experiment. Estrone is not readily soluble in water and the saturation level is reported to be 4 ppm. In the first experiment estrone was placed in solution at 40 ppm by using alkali and then the pH reduced to 8 to make up the stock solution. Both the finely particulate precipitate and the solution were used to make the dilutions. In the second experiment, the estrone was dissolved in water and the resulting solution assumed to be 4 ppm. The subsequent failure to accomplish sex reversal led to the assumption that the suspension or the precipitate resulting from the alkali treatment of the estrone were being absorbed by the eggs.

A third experiment, with fall chinook salmon, is now in progress. In this experiment the technique of estrone dilution used in the first experiment was duplicated and in addition a second phase incorporated to determine if the suspension or the entire precipitate is being absorbed. The fingerling are about of a size for easy sexing. No differential mortality exists between the treated and control lots in any group. There are indications that sex reversal will be accomplished in some of these lots and that it will be possible to determine the optimum concentration and method of estrone preparation from this experiment.

DEVELOPMENT OF METHODS FOR THE REDUCTION OF THE INCIDENCE OF TREMATODE CERCARIAE IN HATCHERY WATER SUPPLIES

Evaluation of prototype electrical grid in water supply

Tests of the electrical grid, designed to electrocute cercariae at a charge of 230 volts per inch and an exposure period of one second, proved effective. The grid was in operation from July 1 to September 19 under operating conditions for which it was designed. During this period, the average cercarial counts increased by 30. Samples of cercariae-free target fish given consecutive weekly exposures in the untreated stream water at no time during this period showed an incidence of infection less than the total accrual of the hatchery fish for the entire period. The tremendous surges of infection encountered in the stream samples were not reflected in the hatchery fish.

The power demand of the grid varied from 55 to 80 kilowatts dependent on the conductivity of the water. A new, more efficient grid using 310 volts per inch and a .4 second exposure period will be installed for test in 1963. This grid will reduce the power demand by 50 percent and should result in a complete cercariae kill.

Exploration of the efficiency of various electrical fields in the destruction of cercariae

This work unit, which resulted in the design of the more efficient electrical grid described above, is now complete. The final report will be combined with that of the prototype evaluation.

In these experiments, tests were conducted using different voltages, frequencies, and exposure times to determine the optimum method of electrocution. The criteria used were percentage kill, capacity, and power demand. On these bases, the 310 volt, 60 cycle, alternating current grid was selected as the most efficient. Tests in frequency were conducted up to one megacycle and in voltages up to 440. The simplicity of installation,

operation, and maintenance in addition to low initial cost make the selected type of electrical grid extremely practicable for hatchery use.

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Mr. Wilmer N. Morgan, Janitor
Mr. Curtis W. Casey, Maintenanceman
Mr. Jack L. Shannon, Maintenanceman
Mr. Emerson L. Jacobson, Maintenanceman

SOUTHEASTERN FISH CULTURAL LABORATORY

Marion, Alabama

Kermit E. Sneed, Chief

Dr. Robert E. Busch who joined our staff on August 24, 1962, is working on genetics, endocrinology and related problems of warmwater fish culture.

Previous research by Dr. Dupree on the vitamins essential for the growth of channel catfish was confirmed.

Electrophoretic separation of the blood proteins of channel catfish fingerlings showed differences in the albumin-globulin ratios associated with diet and stress.

Channel catfish fingerlings did not grow on crystalline amino acid test diets that satisfactorily had grown chinook salmon and rainbow trout at the Western Fish Nutrition Laboratory.

The biological value to channel catfish of two plant proteins, gluten and soybean; and one animal protein, casein; were compared.

Bid invitations were issued for the construction of a laboratory-office combination building. Construction of a "wet" laboratory and at least two artesian wells is planned.

EFFECT OF VARIOUS GONADOTROPIC AND NON-GONADOTROPIC HORMONES ON SPAWNING

As reported previously, human chorionic gonadotropin and fish pituitary when injected alone induced ovulations, and thyroid-stimulating hormone when combined with human chorionic gonadotropin or fish pituitary caused ovulation in the goldfish. However, thyroid-stimulating hormone, pregnant mare serum (PMS), or follicle-stimulating hormone, when used alone, or thyroid-stimulating hormone when combined with follicle-stimulating hormone or PMS did not cause ovulation. Thyroid-stimulating hormone when combined with PMS appeared to ripen the ovaries more than the water which was injected into the control fish.

Adrenocorticotropic hormone, progesterone, thyroid-stimulating hormone, and human chorionic gonadotropin alone and in combination were injected into gravid goldfish. Ovulations were obtained when human chorionic gonadotropin alone was injected or when human chorionic gonadotropin was combined with adrenocorticotropic hormone or progesterone. Human chorionic gonadotropin and thyroid-stimulating hormone with either adrenocorticotropic hormone, or progesterone caused abnormal ovulation. Adrenocorticotropic hormone, thyroid-stimulating hormone, fish pituitary, and follicle-stimulating hormone were injected alone and in combination into gravid channel catfish. Human chorionic gonadotropin and fish pituitary alone induced ovulation as well as human chorionic gonadotropin or fish pituitary combined with thyroid-stimulating hormone or follicle-stimulating hormone. Thyroid-stimulating hormone or follicle-stimulating hormone injected alone did not cause ovulation.

EFFECT UPON SEXUAL MATURATION OF VARIOUS HORMONES

A hormone-pellet experiment to speed up the sexual maturity of the female conducted in 1960 is being repeated on sexually immature goldfish and channel catfish. Seven treatments consist of human chorionic gonadotropin, pregnant mare serum, three pituitary fractions, progesterone, and a pituitary residue, each bound with cholesterol and pressed into pellets. These pellets were inserted into the body cavity of the sexually immature female fish. Cholesterol-only pellets serve as controls.

At the end of 60 days, six goldfish from each of the seven treatments and six goldfish from the control group were examined. The ovarian weights of the fish that received human chorionic gonadotropin, mare chorionic gonadotropin, pituitary fraction No. 1 or pituitary fraction No. 2 were similar to the controls; whereas the ovarian weights of the groups that received either progesterone, pituitary fraction No. 3 or the pituitary residue were much smaller than the controls. Although in the 1960 experiment the ovarian weight of the groups receiving human chorionic gonadotropin,

pituitary fraction No. 1 or pituitary fraction No. 2 were considerably greater than the controls, the hgiher water temperatures employed during the second experiment may have aided the controls in "keeping up" with the human chorionic gonadotropin and pituitary fraction groups.

The channel catfish groups have not been sampled to date.

SEX REVERSAL AND STERILITY

Limited data from fish injected with a progesterone (Norlutin)-cholesterol pellet indicate that Norlutin can effectively retard ovarian development. Four synthetic progesterone compounds (Repromix, Norlutin, Delalutin and Enovid) are being fed to Gambusia and goldfish in an agar-bound diet in an attempt to produce temporary sterility. Current observations suggest that Norlutin and Delalutin are toxic to Gambusia but not to goldfish.

SYNTHETIC DIETS FOR CHANNEL CATFISH

Over 100 synthetic diets containing a number of purified nutrients in varying proportions, bound with CMC or agar, were prepared and tested.

TROUGH FEEDING OF FLATHEAD CATFISH

Preliminary research was completed and results reported.

AMINO ACID TEST DIETS FOR CHANNEL CATFISH

In the 1962 Quarterly Reports we presented the results on our trials of amino acid test diets. In summary, we found that crystalline amino acid purified diets found by the Western Fish Nutrition Laboratory to give satisfactory growth when fed to chinook salmon and rainbow trout would not produce growth in channel catfish. Subsequently, we altered the various components in the diet (type and quantity of binder, mineral mixtures, bulk level, type and level of carbohydrate, and water content) but no growth resulted.

Since the growth response of channel catfish when fed casein (whole protein) is satisfactory, we substituted casein hydrolysate and a different amino acid mixture (ratio of the individual amino acids identical to those found in casein) for the Western Fish Nutrition Laboratory amino acid mixture. In two experiments, it appears that some growth occurred in those groups that received the casein hydrolysate or casein-analysis amino acid mixture. Therefore, we now believe that the problem of little or no growth response may involve an amino acid imbalance (competition between the individual amino acids for absorption through the intestinal mucosa or competition after absorption). With this in mind, new amino acid mixtures are being designed for diets to be fed in 1963.

COMPARISON OF VARIOUS ANIMAL AND VEGETABLE PROTEINS FED TO CHANNEL CATFISH

Two proteins (gluten and soybean) extracted from plants, and one animal protein (casein) were fed at seven levels (11.7, 17.1, 22.4, 27.8, 33.6, 39.1, and 50.2 percent of the diet) to channel catfish fingerlings. These proteins were compounded into purified diets containing a salt mixture, vitamin supplement, corn oil, white dextrin, alpha-cellulose flour, and bound with an agar-water mixture. Growth increased linearly with casein up to 39.1 percent protein, but protein fed above this level (50.1 percent) resulted in less growth. With the casein diets, food conversions were best between 33.6 and 39.1 percent protein. Food conversions with soybean protein and gluten diets were best at 33.6 percent protein.

ELECTROPHORESIS OF CATFISH BLOOD SERUM PROTEINS

Preliminary research is being conducted on the serum proteins of the blood of channel catfish. Differences and similarities in the electrophoretic patterns of fish from a number of environments and of fish subjected to various stresses are being sought. It appears that fish receiving "good" diets and living in "normal" environments have similar blood protein patterns. It also appears that groups of fish

that were diseased, starved or in other ways "stressed" have blood protein patterns different from the controls and from each other.

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

An instrument to measure oxygen and temperature of water has been built and tested. The results of this research were published.

Another instrument that measures oxygen, light, temperature, and conductivity has been built, but a description has not been published as yet.

TRAINING PROJECTS

Students at the Marion in-service training school have as part of their course a "term" problem. Investigation of the following problems, chosen by three of the students, are being directed by our personnel.

1. Comparison of the rates of growth of male and female catfish. Hatchery reared channel catfish will be sexed, weighed, measured, and aged. Groups of fish from the "wild" will be treated similarly. Statistical comparisons of these groups will be performed to determine differences in growth.

2. Study of the similarities and differences in the serum proteins of channel catfish exposed to various stresses. (Preliminary results presented in previous section of this report.)

3. Study of the effect of various progesterone-related steroids in the diet on the reproduction of goldfish and Gambusia. (Preliminary results presented in previous section of this report.)

STAFF

Mr. Kermit E. Sneed, Chief
Dr. Harry K. Dupree, Fishery Biologist
Dr. Robert E. Busch, Geneticist
Mr. Ortus L. Green, Fish Hatchery Manager
Mr. Eugene McCauley, Fish Hatcheryman
Mrs. Mabel A. Jones, Clerk-Stenographer

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

The DDT-cutthroat chronic-effects experiment at Jackson, Wyoming was terminated and the results analyzed.

The 2,4-D-bluegill chronic-effects experiment at Tishomingo, Oklahoma was terminated. Significant pathology resulted from exposure to the herbicide.

Pond and feeding studies on heptachlor and bluegill are under way at Marion, Alabama.

The DDT study at Blackburn Pond near Denver was terminated.

Studies on effects of time and temperature on toxicity to sunfish were continued at Denver. Determinations were also made on the toxicities of many new insecticides.

Equipment for measurements with radioisotopes has enabled us to increase the scope of our work with DDT metabolism and refine our determinations.

Work with microorganisms has resulted in reductions in amounts of chlorinated hydrocarbons held in aquaria.

PEST CONTROL PROGRAMS

Spruce Budworm

In 1962, the U. S. Forest Service conducted a large-scale spruce budworm control program on the Carson and Santa Fe National Forests in New Mexico. Application rate for 1,000 feet on each side of major streams was 1/2-pound per acre, but the major portion of the area received 1 pound per acre.

Our study of the effects of the spray on fish and aquatic invertebrates was limited to the Pecos River and the Rio La Junta. Observations of fish in the streams and in live-cars indicated the fish suffered no acute effects. Large numbers of aquatic insects were killed by the DDT spray on the Rio La Junta, even though DDT was found in only 3 of 13 samples and the greatest amount of DDT detected in the water was only 2.7 ppb. Peak numbers of dead and dying insects were found in the water between the first and second hour after the sprays.

On the Rio La Junta, samples of cutthroat trout had their highest residues, 4.2 ppm of the DDT complex, at the 31-day time, compared with earlier and later collections. In the Pecos River drainage, brown trout taken 18 days after the spray had up to 5.2 ppm of DDT and its derivatives, whereas none of the pre-spray samples contained more than 0.5 ppm.

EXPERIMENTAL FIELD STUDIES

DDT and Cutthroat Trout

Studies on chronic effects of DDT on cutthroat trout began at Jackson, Wyoming in December, 1960 and were terminated in August of 1962. 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 exposed to DDT once a month in bath form, and one lot was neither fed nor exposed to DDT. Samples were withdrawn at intervals for chemical analysis for DDT and its metabolites, and for histological examination. Records were kept on fish size and on day-to-day mortalities in the various lots.

Residues in the trout continued as reported a year ago, with largest amounts of DDT, DDD, and DDE in the fish exposed to the highest treatments, i.e., fed 3 and 1 mg. per kilogram of body weight or exposed to DDT in baths at 1 and 0.3 ppm.

Differences in weight among lots of fish continued as before, with greatest weights showing in the survivors of the strongest treatments.

Histopathology studies on these fish revealed no pathology attributable to DDT.

Microhaematocrit measurements made on the trout throughout the experiment showed no differences between treated and untreated fish.

Herbicides and Bluegill Sunfish

An experiment to measure chronic effects of 2,4-D on bluegill sunfish in treated ponds was begun at Tishomingo, Oklahoma in July 1961. Three ponds were partitioned with polyvinyl chloride sheeting to provide six testing spaces for fish. Each subdivision measured 1/10 acre. One space was used for the untreated control, and each of the others was treated with one of five concentrations of Esteron 99, propylene glycol butyl ether ester of 2,4-D, the treatment concentrations being 10, 5, 1, 0.5, and 0.1 ppm.

The herbicide remained in the ponds near treatment concentrations for about three weeks, and all disappeared from the water after 12 weeks.

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

Mortality among fish was 19 percent in the 10 ppm pond in the first week, but there were essentially no deaths in the other ponds. Applications at the same rates in adjacent ponds in 1962 resulted in 100 percent mortality at 10 and 5 ppm. Spawning was delayed for two weeks in the 10 ppm pond; all other lots spawned at the normal time. Fry production appeared to be essentially the same in all lots.

Whole body analyses of fish for 2,4-D were performed, and the results are reported in table 1. Residues were found in fish from only the ponds with the two highest concentrations, and in relatively small amounts.

Histopathological examination of sampled bluegills was made by Dr. E. M. Wood, and the findings were described. Three kinds of pathology were found in the fish, involving the liver, vascular system, and brain. Liver glycogen was markedly depleted, accompanied by shrinkage, irregular staining characteristics, and loss of normal morphology of liver parenchyma cells. Globular deposits suggestive of glycoproteins appeared throughout the vascular systems. A marked stasis and engorgement of the circulatory system of the brain occurred. Surviving fish apparently recovered completely, and after 112 days no pathologic changes were seen.

Microhematocrit readings did not differ among the lots of fish.

During the spring months the nine 0.01-acre pools on the levee at La Crosse, Wisconsin were prepared for use. Vinyl plastic liners were installed to reduce reactions between the new concrete and herbicides. Loam was spread to a depth of 6 inches to serve as bottom material. Sprigs or tubers of 14 species of aquatic plants were planted in the bottoms. The pools were filled to a depth of 3 feet with well water, and inoculations with phyto- and zooplankton were made.

Electric service to the levee was installed and a 40-W. incandescent bulb was mounted over each pool. The lights attracted swarms of mayflies and midges which emerged from the rivers and bass pond. The insects served as food for the fish and contributed to the establishment of bottom fauna.

DDT in Blackburn Pond

A 1/2-acre pond on the Blackburn property near Denver was treated with DDT at the rate of 0.02 ppm in July 1961 for a study on the breakdown and distribution of the compound in a warm-water pond. Sampling of the existing fish population, rainbow trout and bullheads, and of water, crayfish, aquatic vegetation, and bottom sediments was carried on after the application of DDT, and the samples analyzed for residues of the insecticide and its metabolites. Sampling was continued until the termination of the study in November 1962.

Table 1:--Amounts of 2,4-D, in ppm, measured in whole bodies of bluegill exposed to Esteron 99 on July 4, 1961 at Tishomingo, Oklahoma

Date of sample	Pond 11 Treated at 10 ppm	Pond 12 Treated at 5 ppm	Pond 21 Treated at 1 ppm	Pond 22 Treated at 0.5 ppm	Pond 31 Treated at 0.1 ppm	Pond 32 Control
(1961)						
July 3	ND[1/]	ND	ND	ND	ND	ND
5	2.0	1.0	ND	ND	ND	ND
7	1.6	0.3	ND	ND	ND	ND
18	ND	ND	--	--	--	--
Aug. 1	ND	ND	--	--	--	--
29	ND	ND	--	--	--	--

1/ ND denotes no detectable 2,4-D.

The concentration of DDT in the pond water was highest 30 minutes after treatment. A decline in DDT levels then took place; none could be detected 21 days later. Aquatic vegetation contained 6 to 30 ppm of DDT + DDE + DDD in the first week after treatment which 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 lower.

Bullheads and trout contained the greatest amounts of chlorinated hydrocarbon 30 to 40 days after treatment, with concentrations over 4 ppm. Levels slowly declined after that, averaging 3.5 ppm in samples taken 9 and 10 months after treatment in both species, and 3 ppm in rainbow trout taken 14 months after treatment. Crayfish developed lower DDT residues than did trout, and contained 0.33 ppm after 14 weeks.

Each pool was stocked with 300 subadult bluegills from the Lake Mills National Fish Hatchery and with 88 adult bluegills from the Mississippi River more than a month before a herbicide was applied. Some losses due to Saprolegnia occurred despite careful screening, handling, and prophylaxis.

Philip A. Gilderhus took charge of these investigations in August. The first treatments with sodium arsenite (40 percent active $NaAsO_2$) were made during the second week of August. The rates and times of applications differed from pool to pool. Pool No. 6, designated as a control, was not treated with herbicide. The total concentrations received by eight pools before mid-October ranged from 0.8 to 24 ppmv.

Fish:--Mortalities were observed during the sampling season but most of the dead were not recorded because they sank out of sight to the bottom. The heaviest mortalities occurred in pools which received 10 and 24 ppmv of herbicide. The survivors in these pools showed lower gains in weight than fish in pools which received less than 1 ppmv.

Bottom fauna:--Midge larvae were the only bottom organisms present in sufficient numbers to permit an estimate of herbicidal influences. In general, there were larger numbers of larvae in pools which received the lower concentrations of sodium arsenite. Shortcomings appeared, however, in the sampling techniques which will be corrected before the 1963 season.

Rooted plants:--The introduced plants, with the exception of Sagittaria, failed to grow in the pools. The heavy blooms of phytoplankton were believed to be responsible.

Residues:--A silver diethyldithiocarbamate method was adapted for assay of arsenic residues in water, fish, and bottom fauna. It is a more simple and reliable technique than others, and it can be modified to detect either small or large concentrations of arsenic.

The assays for residues were begun in December. An analysis of water from a pool which received one treatment of 10 ppmv revealed that 43.7 percent of the arsenic remained after 8 weeks.

After final samples were taken in mid-October, the pools were drained and the fish were removed. Small specimens were weighed, measured, and frozen for future assays of arsenic

residues. Adult fish were marked and moved to the holding house with the intent of evaluating their reproductive successes in 1963.

The fish and crayfish in Blackburn Pond showed unusually high proportions of DDD (50-60 percent of the total chlorinated hydrocarbons in some fish after 12 months), compared with the pattern seen in other studies.

Organs and tissues of bullheads taken 14 months after treatment showed the following amounts of the DDT complex, in ppm: brain, 10.1; skeletal muscle, 0.5; gut, 1.9; liver, 0.9; fat, 38.8; ovary, 0.2; and blood, 5.0. DDD was found in relatively great amounts in all tissues and organs except brain and blood.

LABORATORY STUDIES

Toxicant tolerance

During 1961 and 1962 the laboratory conducted many bioassay tests to establish toxicities and tolerances of different species of fish. Tables 2 and 3 present results for some insecticides, herbicides, and fungicides.

Toxicity, time, and temperature

Studies were conducted on the influence of time and temperature on the toxicity of heptachlor, kepone, and malathion to sunfish. Bridges (in press) has reported on the results on heptachlor and kepone, and his abstract is as follows:

The toxic effects of heptachlor and kepone were measured by the determination of the Median Effective Concentration, or EC_{50} (the concentration required to produce 50 percent mortality of the fish), at each time and temperature tested. EC_{50} values for heptachlor ranged from 0.017 mg/1 for 96 hours exposure at 75° F. to 0.092 mg./1 for 24 hours exposure at 45° F.; comparable values for kepone were 0.044 mg./1 and 0.62 mg./1. The toxicity of kepone greatly increased with the time of exposure, whereas the influence of increased time on the toxicity of heptachlor was only moderate. Higher temperatures caused a moderate increase in toxic effects of both compounds.

The work with malathion was done with bluegill sunfish, and the results are presented in table 4. Time and temperature made a moderate difference in toxicity.

Microorganisms and chlorinated hydrocarbons

Work was initiated or continued in 1962 to learn of the effects of certain species of bacteria on chlorinated hydrocarbons in water. This laboratory is interested in methods of altering the toxicity or the structure of pesticides so that hazards to fish may be reduced. The possibility of degradation of DDT, toxaphene, and endrin was explored in aquaria in the laboratory as a preliminary to possible future investigation.

Five species of bacteria were used, Micromonospora chalcea, Pseudomonas aeruginosa, P. fluorescens, P. stutzeri, and Corynebacterium pyogenes. As shown in table 5, the concentration of endrin was apparently reduced by C. pyogenes but not by P. aeruginosa or M. chalcea. As table 6 shows, C. pyogenes appeared to eliminate toxaphene, while P. aeruginosa and M. chalcea reduced its concentration. With DDT, all organisms except P. stutzeri greatly reduced the concentration in 7 to 16 days; table 7 shows the effects of C. pyogenes on DDT.

The larger amount of bacterial culture eliminated the DDT at a faster rate than did the smaller amount.

Studies with radioactive DDT, water, and C. pyogenes indicated that a high proportion of the DDT present was taken up by the bacterial cells, or by contaminating protozoans which were present, and that one or both microorganisms apparently metabolized DDT to DDD and possibly to DDE.

DDT in fish eggs

A survey was begun to ascertain the amounts of DDT and its derivatives found in salmonid eggs in National fish hatcheries. Eggs of all species have not yet been received, but DDT has been found in all six species analyzed thus far. Table 8 presents the results of analyses.

Table 2:--Toxicity measurements of various insecticides versus rainbow trout, bluegill, and redear sunfish.

Toxicant	Species	Wt. or Length	Temp. °F.	Estimated EC_{50}, µg per l.[1/]		
				24 hr.	48 hr.	96 hr.
Aldrin, Tech.	Bluegill	0.6 g.	65	10	6	-
Allied GC 3707 Tech.	"	0.4 g.	75	600	500	-
Allied GC 3707, WP	Rainbow	0.6 g.	65	95	-	-
Allied GC 3707, EC	"	0.6 g.	65	170	-	-
Allied GC 4072, Tech.	Bluegill	0.4 g.	75	3	-	-
DDT, p-p'	Rainbow	2-3 in.	55	18(18 hrs.)	11(32 hrs.)	10(56 hrs.)
DDT, p-p'	"	2-3 in.	55	10	8	7
DDT, p-p'	"	0.6 g.	65	10	-	-
DDT, p-p'	"	0.4 g.	65	6	-	-
DDT, p-p'	Bluegill	1.5 g.	75	5 - 6	-	-
DDT, p-p'	"	0.4 g.	75	6	-	-
DDT, p-p'	Redear	3 g.	75	19	15	-
DDT, anti-resistant, 25% EC	Rainbow	2-3 in.	55	10	-	-
DDT, anti-resistant, 25%, oil	"	2-3 in.	55	10	-	-
DDT, anti-resistant, 50% WP	"	2-3 in.	55	24	21	16
DDD(TDE) Tech.	"	2-3 in.	55	30	-	-
DDVP, Tech.	"	2-3 in.	55	500	-	-
Dibrom	"	2-3 in.	55	80(18 hrs.)	-	-
Dibrom, Tech.	"	2-3 in.	55	70	-	-
Endrin, Tech.	Bluegill	0.4 g.	75	0.4	-	-
Endrin, Tech.	"	0.6 g.	75	0.4	-	-

1/ Times of exposure are indicated in parentheses where they deviate from times in column heads.

Table 2. (continued)

Toxicant	Species	Wt. or Length	Temp. °F.	Estimated EC_{50}, µg per l.[1/] 24 hr.	48 hr.	96 hr.
Fairfield's 279	Rainbow	0.5 g.	55	360	-	-
Fairfield's OT 60-6	"	0.8 g.	55	100	-	-
Heptachlor, 2% granular	"	2-3 in.	55	150	90	70
Hercules 7175, Tech.	Bluegill	0.4 g.	75	40,000	-	-
Hercules, 7531, Tech.	"	0.4 g.	75	25,000	-	-
Kelthane, Tech.	Rainbow	0.7 g.	55	110	-	-
Kepone	Bluegill	2 in.	65	380(18 hrs.)	240(32 hrs.)	110(56 hrs.)
Malathion, Tech.	Rainbow	2-3 in.	55	100	-	-
Malathion, Tech.	Redear	3 g.	75	170	100	60
Malathion, Tech.	Bluegill	0.4 g.	75	45	35	-
Malathion, Tech.	"	0.6 g.	75	120	-	-
Methyl parathion, Tech.	"	0.6 g.	75	8,500	-	-
MGK's Evergreen	Rainbow	0.4 g.	55	800	-	-
MGK's 6103	"	0.5 g.	55	150	-	-
MGK's 6243	"	0.8 g.	55	750	-	-
Methoxychlor, Tech.	"	0.7 g.	55	20	-	-
Phosphamidon	"	2-3 in.	55	5,000(18 hrs.)	-	-
Sevin, Tech.	"	2-3 in.	55	3,500	2,000	-
Stam F-34, Tech.	"	2-3 in.	55	-	4,000	-
Zectran	"	2-3 in.	55	7,000(18 hrs.)	-	-

1/ Times of exposure are indicated in parentheses where they deviate from times in column heads.

Table 3:-- Toxicity measurements of various herbicides, fungicides and antibiotics versus rainbow trout, bluegill, and redear sunfish.

Toxicant	Species	Wt. or Length	Temp. °F.	Estimated EC_{50}, µg per 1. 1/		
				24 hr.	48 hr.	96 hr.
Amchem's silvex	Bluegill	2 in.	65	700(18 hrs.)	600(32 hrs.)	-
Amitrol-T	"	2 in.	65	(no mortality at 10,000 µg/1 over 100 hrs.		
Antimycin A	Rainbow	2-3 in.	55	0.25(18 hrs.)	-	-
Casaron	Redear	3 g.	65	(no mortality at 20,000 µg/1 at 48 hrs.)		
Diquat	Rainbow	2-3 in.	55	(no mortality at 10,000 µg/1 over 100 hrs.)		
Esteron 99	Bluegill	2 in.	65	1,200 (18 hrs.)	-	-
Esteron 99, EC	"	0.6 g.	75	700	-	-
Fenac, sodium salt	Redear	3 g.	75	(no mortality at 12,000 µg/1 at 48 hrs.)		
Fenac, " "	Rainbow	0.6 g.	65	10,000	7,500	-
Kurasol	Bluegill	0.6 g.	75	120,000	-	-
Weedar MCP	"	2 in.	65	(no mortality at 10,000 µg/1 over 100 hrs.)		

1/ Times of exposure are indicated in parentheses where they deviate from times in column heads.

Table 4:--EC_{50} values for malathion and bluegill, in milligrams per liter.

Temperature °F.	6 hours	12 hours	24 hours	48 hours	96 hours
45	1.04	0.52	0.28	0.16	0.087
55	0.80	0.54	0.22	0.11	0.084
65	0.56	0.22	0.135	0.11	0.055
75	0.35	0.20	0.124	0.086	0.040
85	0.16	0.078	0.07	0.044	0.020

Table 5:--Amounts of endrin, in ppm, remaining in aquaria after exposures to bacteria. Amount of endrin added before experiment was 0.1 ppm.

Time	Control	C. pyogenes	P. aeruginosa	M. chalcea
Pre-	0.057	0.057	0.061	0.055
1 day	0.062	0.062	0.062	0.062
3 days	0.078	0.037	0.065	0.062
8 days	0.052	0.033	0.048	0.045
14 days	0.064	0.036	0.033	0.037
17 days	0.062	0.035	0.05	0.057
24 days	0.069	0.042	0.055	0.073
31 days	0.074	0.047	0.058	0.069

Table 6:--Amounts of toxaphene, in ppm, remaining in aquaria after exposures to bacteria. Amount of toxaphene added before experiment was 0.1 ppm.

Time	Control	C. pyogenes	P. aeruginosa	M. chalcea
Pre-	--	0.079	--	--
2 days	0.082	0.018	0.082	0.027
4 days	0.05	0.0029	0.028	0.026
9 days	0.06	0.008	--	0.032
15 days	0.062	0.0054	0.0032	0.031
22 days	0.068	0	0.006	0.03

Table 7:--Amounts of DDT, in ppm, remaining in aquaria after exposure to C. pyogenes. Amount of DDT added before experiment was 0.1 ppm.

Time	Control	5 ml. C. pyogenes	15 ml. C. pyogenes
Pre-	0.016	0.027	0.03
1 day	0.021	0.020	0.010
4 days	0.012	0.0085	0.0025
6 days	0.0016	0	0

Table 8:--Amount of DDT, and derivatives ppm, measured in eggs from National fish hatcheries, November and December, 1962.

Species	Source	DDD	DDT	DDE	Total
King Salmon	Little White Salmon, Washington	0.08	0.05	0.24	0.37
Red Salmon	Leavenworth, Washington	0.07	0.05	0.23	0.35
Brown Trout	Cortland, N. Y.	0.06	0.04	0.20	0.30
Lake Trout	Pendill's Creek, Michigan	0.04	0,03	0.08	0.15
Rainbow Trout	Manchester, Iowa	0.17	0.12	0.14	0.43
Brook Trout	Berlin, N. H.	Trace	Trace	0.10	0.10

Analyses for other insecticide residues showed no other chlorinated hydrocarbons to be present.

EQUIPMENT, METHODS, AND TECHNIQUES

Plastic film and herbicides

The use of plastic films in herbicide research has been important to this laboratory because of the possible use of plastic bags for test containers and the fabrication of plastic barriers across ponds to contain herbicides during field tests. Bridges and Sanders (in press) have described studies made in the Denver laboratory and have given results of measurements of the permeation and retention of herbicides in water in contact with plastic films.

Tests with various herbicides and polyethylene and saran films showed that some herbicides diffuse through these materials in aqueous situations. Other tests with polyvinyl chloride film showed that it is an effective barrier for propylene glycol butyl ether esters of 2,4-D and for the butoxyethanol ester of silvex.

Analysis of malathion

Kallman authored an article on the microdetermination of malathion by photometric means. 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 to be specific for esterified carboxyl groups; several organophosphorus compounds not possessing such groups do not react similarly when tested.

STAFF

Dr. Oliver B. Cope, Chief
Dr. Burton J. Kallman, Chemist
Mr. Walter R. Bridges, Fishery Biologist
Mr. Charles C. Van Valin, Chemist
Mr. Donald T. Allison, Fishery Biologist
Mr. Paul J. Frey, Fishery Biologist
Mr. Herman O. Sanders, Entomologist
Mr. John J. O'Donnell, Jr., Physical Science Technician
Mr. Ronald E. Elkin, Jr., Fishery Biologist
Mr. Austin K. Andrews, Fishery Biologist
Mr. Philip A. Gilderhus, Fishery Biologist
Miss Ellen M. Roeder, Biological Aid
Mr. Edward L. Davis, Fishery Aid
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Mr. Ernest A. Giedd, Physical Science Aid
Mr. Joseph P. McCraren, Fishery Aid
Mr. Henry T. DeHoll, Physical Science Aid

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

The Fish Control Laboratory was dedicated on October 25.

The bioassay facilities were expanded to accomodate about 800 tests per week. Controlled temperature tanks were installed. Procedures for basic and intensive assays were defined and tested.

Selected groups of chemicals including rotenoids, cresols, chlorinated hydrocarbons, organophosphates, pesticide mixtures, pharmaceuticals, chemo-sterilants, endothal derivatives, and Antimycin were screened.

Nearly one million fish were acquired from hatcheries and natural waters. Numerous problems in care, feeding, handling, disease, and stress were encountered.

The physiology laboratory was staffed and equipped. The new aquarium was open to the public from June through September.

Development of facilities at the Warm Springs, Georgia, laboratory was started. A semi-field project was staffed and equipped.

DEDICATION

Commissioner Clarence F. Pautzke dedicated the Fish Control Laboratory on October 25 at a formal ceremony which was sponsored by the Greater La Crosse Chamber of Commerce. Other speakers at the event included Mayor Milo G. Knutson; Director Lester P. Voigt, Wisconsin Conservation Department; Executive Vice President Richard H. Stroud, Sport Fishing Institute; Regional Director Robert W. Burwell, BSFW, Minneapolis; and Regional Director W. Fent Carbine, BCF, Ann Arbor. Open House at the Laboratory for guests and the public followed the ceremony.

Through the courtesy of the Wisconsin Conservation Department, the dedication was included as a part of the Sixteenth Tri-State Fishery Conference which was held in La Crosse on October 24 through 26. Approximately 250 participants came from Michigan, Illinois, Wisconsin, Iowa, and Minnesota. Some attractive exhibits of fish and fishery gear of interest to biologists and the public were set up at the laboratory by the Wisconsin and Minnesota conservation departments.

SCREENING OF CHEMICALS

A systematic program of screening was inaugurated following an expansion of facilities and thorough shakedown trials of equipment and methods. Up to 500 tests of 48- or 96-hours duration are performed per week in 1-gallon, disposable, glass vessels. Rainbow trout, goldfish, black bullhead, and bluegill of 2 to 4 inches in length are exposed to each chemical at concentrations of 0.01, 0.10, 1.0, and 10 ppm in standard reconstituted water at 12° C. The reactions of the fish are observed at 0.75-, 1.5-, 3-, and 6-hour intervals following an application of chemical, and at 24-hour intervals thereafter.

Thus far, 105 compounds have been selected and solicited from 22 manufacturers. They included formulations of rotenone, isomers of cresylic acid, chemosterilants, chlorinated hydrocarbon and organophosphate insecticides, psychotomimetrics, bactericides, algicides, fungicides, parasiticides, herbicides, lampricides, pharmaceuticals, anesthetics, and sedatives. Some were compounds which showed promise as fish toxicants in bioassays at Leetown, West Virginia, and Hammond Bay, Michigan.

Most of this select group warranted further testing beyond first screening, and they were subjected to delineative screening to determine concentrations which produce all-or-none effects. Whenever the range within bracketing concentrations met practical requirements, the compounds were moved into the intensive screening program.

INTENSIVE SCREENING OF CHEMICALS

The capacity for screening in this project is 180 tests per week in 5-gallon

vessels and up to 320 tests per week in 1-gallon vessels. The progress of a chemical through intensive screening involves five main steps: against a variety of target, sport, and forage fishes; against other aquatic forms; at various water temperatures; in waters of different qualities; and in semi-field conditions in plastic pools.

The target fish include gar spp., bowfin, gizzard shad, goldfish, carp, sucker spp., bullhead spp., green sunfish, pumpkinseed, yellow perch, and freshwater drum. The sport fish used in experiments include trout spp., northern pike, channel catfish, largemouth bass, bluegill, and walleye. The forage fish are represented in tests by central mudminnow, minnow spp., sucker spp., killifish spp., mosquitofish, brook stickleback, and silverside spp.

A compound which shows promise as a fish control agent is tested against phyto- and zooplankton, higher aquatic plants, bottom fauna, macro-crustaceans, amphibians, and reptiles. Its effects on fish disease organisms, including bacteria, fungi, and parasites, are also observed.

Electrical service and apparatus for control of water temperatures in bioassay troughs were installed and put into use during the year. The temperatures selected were 12°, 17°, 22°, and 27° C. Equipment for 2° and 7° will be added later. Variations in water quality are obtained by addition or subtraction of components in the reconstituted deionized water.

Facilities for semi-field tests of the more promising chemicals were provided by erection of 18 vinyl plastic wading pools on the levee. Sand or loam bottom materials are added, aquatic plants are established, and filtered pond water is used. The biological activity of the test compound is observed, its rate of degradation is determined, and the fate of residues is studied.

Many problems were encountered and investigated before the intensive program was operational. Many species of fish were tested for their suitability as bioassay animals, including such aspects as: their oxygen requirements with respect to the load capacity of test vessels; water quality requirements; feed requirements; their tolerances for holding and acclimatization; their susceptibilities to bacterial, fungal, and parasitic diseases; and their responses to prophylatic and therapeutic agents and procedures. Methods for the detection and evaluation of stresses in experimental fish were investigated, particularly those stresses which are produced by chemicals, diseases, and electric shock. An electrostimulator was developed to enable observers to detect when fish in bioassay vessels are actually dead.

There was also the necessity of developing or refining analytical methods for chemistries of test waters, of fish blood, and of residues of toxicants. Instruments were acquired, calibrated and adapted to use in the screening program. Equipment was procured or developed to increase or improve the supply and circulation of water, the supply and distribution of compressed air, the capacity and distribution of electrical power, and to handle and dispose of bioassay vessels and specimens. Many innovations were made during the year to improve operations, to enhance safety for personnel and facilities, and to achieve economies in labor.

Rotenoids

Nine commercial preparations of rotenone were subjected to intensive testing at 12°, 17°, and 22° C. with up to nine species of fish. There was little difference in performance at 22° C. by all formulations, but their toxicities were greatly and differentially affected by lowered temperatures. Only two products were reasonably effective on all test fishes at 12° C. The differences in performance were less apparent on such sensitive species as rainbow trout, bluegill, and largemouth bass.

Cresols

Three isomers of cresylic acid were tested against seven species of fish at 12°, 17°, and 22° C. Differences in temperature did not appear to influence relative toxicities.

The para- isomer was the more toxic, followed in order by ortho- and meta- isomers. It was learned that the concentrations which produced narcosis are not far removed from lethal concentrations.

Chlorinated hydrocarbons

Several insecticides of this group were subjected to intensive screening. In particular, the concentrations of chlordane, toxaphene, thiodan, aldrin, dieldrin, and endrin which produce all-or-none effects were investigated. A foreign, MS student at Iowa State University performed elaborate tests on toxaphene at the Laboratory from June through August. His final report is not yet available.

Organophosphates

Some of these insecticides have selective toxicities to fish. Screening was begun only after extensive precautions were taken to safeguard personnel because these compounds are extremely toxic to mammals.

In general, the organo-phosphates appear to be more toxic to sunfish than to goldfish and catfish. For example, one agent among a group of coded compounds demonstrated a 100-fold difference in toxicity between catfish and sunfish. The addition of an emulsifier to another agent increased its toxicity substantially, but we have not established whether this was an additive or synergistic effect.

Pesticide mixtures

Combinations of organic phosphates and halogenated insecticides are powerful toxicants with some degree of selectivity. The Ortho Selective Fish Thinner which is made up of malathion and dibrom is much more toxic to rainbow trout, northern pike, bluegill, and largemouth bass than to goldfish, golden shiner, and black bullhead (table 1). The mixture was more toxic than the individual components.

Pharmaceuticals

Substances which have sedative or narcotic properties were selected for testing. They included cyclobutane and cyclohexane methylated preparations. Their direct effects on fish as well as their potentialities as aids or additives in electrical and chemical control are under investigation.

Further tests are underway on some compounds because of indications that they may be useful in the control of fish diseases. Also, psychotimimetrics such as the ergotine alkaloids were observed to influence the behavior patterns of test fishes.

Derivatives of endothal

The broad spectrum of biological activities of endothal derivatives such as bactericides, fungicides, algicides, and herbicides was examined. Some of these activities have possibilities as dual management tools for control of fish, fish diseases, or aquatic weeds. There is also a wide variation in the toxicities of the derivatives to fish. Certain promising combinations of these compounds with other biologically-active chemicals are under investigation.

Chemosterilants

Preliminary investigation of aziridinyl phosphonitriles was begun. Several samples of Apholate were included in intensive tests, and results on fish have varied widely. The inconsistencies in performance were due, we believe, to polymerization in samples and to a suspect solvent. Additional testing with the compound, however, is warranted.

Antimycin

The major emphasis in the intensive screening program was devoted to Antimycin as a potential fish toxicant. The material is an extract of a synthesis produced by Streptomyces spp. It is a powerful fungicide and bactericide, and its toxicity to fish is measured in parts per trillion. The minute quantities needed and its rapid breakdown in water are its principal merits as a possible tool in fishery management.

Significant degradation of Antimycin occurred within 24 hours in bioassay tests.

Table 1:--The all-or-none survival of seven species of fish exposed to Ortho Selective Fish Thinner for 24 hours.

Species	Survival to toxicant (in ppm)					
	at 12° C.		at 17° C.		at 22° C.	
	All	None	All	None	All	None
Rainbow trout	0.01	0.10	-	-	-	-
Northern pike	0.01	0.04	-	-	-	-
Goldfish	6.00	> 6.00	5.00	< 10.00	4.00	8.00
Golden shiner	-	-	-	-	> 1.00	5.00
Black bullhead	< 4.00	6.00	2.00	< 6.00	2.00	4.00
Bluegill	0.08	0.50	0.04	0.10	0.01	0.10
Largemouth bass	-	1.00	0.50	0.80	0.10	0.20

Lethal exposure times appear to be quite short for most of 10 fishes tested. Black bullhead and yellow bullhead were much more resistant to the compound than other species. Those fish which survived the 96-hour bioassays, however, appeared to be especially susceptible to an unidentified, fungus-like disease organism.

The tiger salamander (Ambystoma tigrinum), a serious predator on fish in some hatchery ponds, proved immune to relatively high concentrations of Antimycin.

Pseudo-field tests

The eighteen plastic pools were employed exclusively this year for semi-field tests on Antimycin. All were filled with filtered pond water. One-half of them had sand bottoms; the remainder had loam-bottoms. Rooted aquatic plants were placed in four pools with sand bottoms and in four pools with loam-bottoms. Cultures of plankton were stocked in four sand-bottom pools and in four loam-bottom pools. Two pools served as controls. Bottom faunas which consisted mostly of tendipedids became established naturally because of the proximity to the bass pond and rivers.

Adult goldfish, black bullhead, bluegill, and yellow perch were stocked in each pool at pre-determined rates of loading. In addition, fingerling goldfish, golden shiner, and largemouth bass were stocked within bioassay cages placed in each pool. Following a period of acclimatization during which samples of the water, plankton, and bottom fauna were collected, Antimycin was applied in concentrations of 5 and 10 ppb. In a later trial, the concentrations were 20 and 40 ppb. Observations on the fish kill were made at 24, 48, 72, 96, 216, and 336 hours after treatment.

The Antimycin produced distinctly different results in the loam- and sand-bottom pools. In general, the mortalities of fish were much greater in the loam-bottom pools. The black bullhead, however, was resistant to 5 and 10 ppb of toxicant in all pools. Adult goldfish survived both concentrations in the sand-bottom pools. The fingerling fish in the bioassay cages suffered greater mortalities than adults in the pools.

The second trial with Antimycin at 20 and 40 ppb included adult-size rainbow trout, yellow bullhead, and green sunfish; and fingerling-size goldfish, golden shiner, bluntnose minnow,

and yellow perch. Again mortalities were greater in the loam-bottom pools but the range of differences was smaller than at lower concentrations. The yellow bullhead in pools of both bottom types survived 20 ppb and had less than 10 percent mortality at 40 ppb. All fingerling goldfish in loam-bottom pools were killed by 40 ppb of toxicant, but 7.5 percent survived in the sand-bottom pools.

Many of the fish which survived the first 96 hours of exposure to the toxicant became infected with the unidentified fungus growth and perished. No toxic effects by the Antimycin were detected on phytoplankton and on higher aquatic plants. Blooms of plankton consisting of various flagelated algae, protozoans, and crustacea were common in all pools during the test periods. The bottom fauna did not appear to be affected either.

The degradation of the Antimycin in the pools was very rapid. The more rapid rate was observed in the sand-bottom pools because of their higher alkalinity. This probably accounts for the greater survival of fish in these pools.

Selected fish which were killed within 96 hours by the toxicant were rushed to the Wisconsin Alumni Research Foundation in Madison for mammalian toxicity tests. The results of feeding tests with rats were negative, and the foundation reported that no toxic residues were found in the fish.

Arrangements were made with the foundation in December to continue work with Antimycin in Madison and La Crosse and in the field during 1963.

Physiological Investigations

Richard A. Schoettger and Jeannette R. Ernest were employed in the summer to staff the new Physiology Laboratory. Much of their time during the remainder of the year was devoted to acquiring equipment, testing it, and getting the laboratory into operation.

Aspects of fish hematology are under investigation to determine which of them might be used as indices of physical well-being. Indices are badly needed for routine evaluation of the suitability of fish for bioassays.

A preliminary comparison was made of values obtained by YSI Electronic Hematocrit and microhematocrit-centrifugation methods. Blood samples from severed peduncles of seven species of fish were collected in heparinized (10 percent sodium heparinate) microcapillaries. The fish, 2 to 4 inches long, were taken randomly from bioassay stocks. The results are summarized in table 2. Work is continuing to refine the procedures and to establish statistical differences, if any, between the two methods. The YSI method is slower than the standard microhematocrit, but it appears to give more consistent results.

Determinations of hematocrit and hemoglobin in additional species held under various conditions are in progress. Values from wild fish are to be included. Also, measurements of pH and differential cell counts in fish blood were started.

Studies on gases in fish blood were initiated. A Natleson Microgasometer was employed to measure oxygen and carbon dioxide in blood samples. The apparatus appears to be suitable for both gases. The determinations of oxygen, however, are slow and there are a number of problems related to the introduction of samples and to the cleanliness of glassware. A comparison of the microgasometer and Van Slyke apparatus is in progress.

Warm Springs, Georgia

A $97,492 contract was awarded in May for the construction of a laboratory building and water supply structures. Additional orders for trenching, draining, and filling were issued at later dates. Preliminary plans for a holding house and an auxiliary water supply were drawn and referred to the Branch of Engineering for immediate action. A contract for these facilities should be awarded in the spring of 1963.

With the cooperation of the Branch of Fish Hatcheries, space and facilities are available on hatchery grounds for some

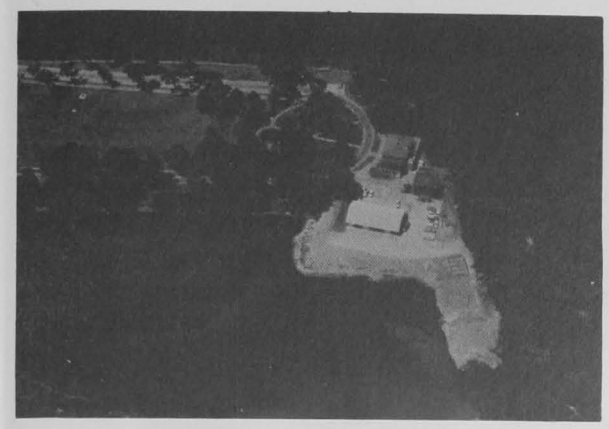


Figure 1

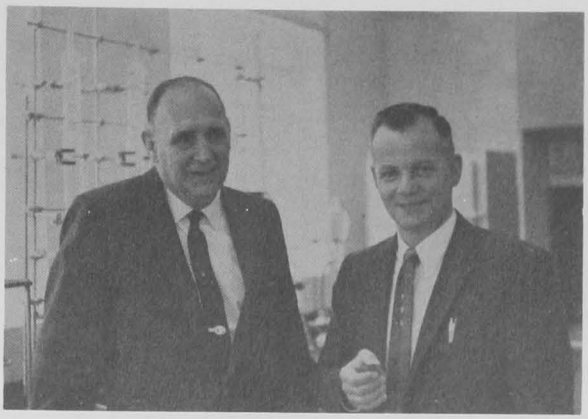
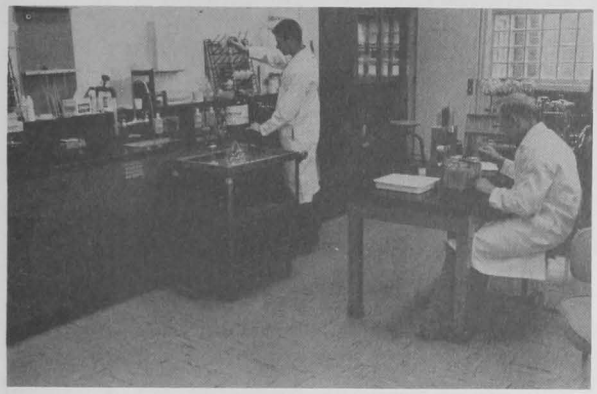


Figure 2



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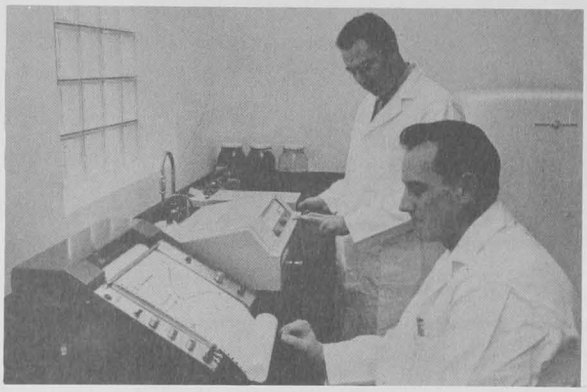

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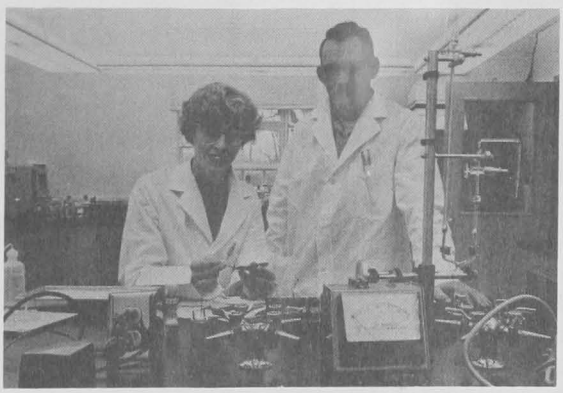


Figure 5

Figure 1:--Fish Control Laboratory, La Crosse, Wisconsin, June 2, 1961.
Figure 2:--Commissioner Pautzke and Dr. Robert E. Lennon at Fish Control Laboratory. Photo courtesy Minneapolis Tribune.
Figure 3:--Chemist Leif L. Marking (standing) and Physical Science Technician Arnold M. Julin preparing compounds for bioassay in the Chemistry Laboratory.
Figure 4:--Biochemist Charles R. Walker (standing) and Physiologist Richard A. Schoettger checking a new DB Spectrophotometer in the Special Use Laboratory.
Figure 5:--Physiologist Jeannette R. Ernest and Richard A. Schoettger engaged in hematology of fishes in the new Physiology Laboratory.

Figure 7

Figure 8

Figure 9

Figure 10

Figure 11

Figure 12

Figure 7:--Physical Science Technicians Arnold M. Julin (left) and Bernard L. Berger record observations on bioassays in Wet Lab (Tank Room). Chemist Leif L. Marking in background. The vessels and troughs in the foreground are employed for basic screening of chemicals.

Figure 8:--The Water Conditioning Room with water softener, charcoal filters, and diatomaceous earth filter to the right of Fishery Biologist Phillip S. Parker and a mixed-bed deionizer to the left. All water used in the bioassays is filtered, deionized, and reconstituted to a prescribed mineral content.

Figure 9:--Biochemist Charles R. Walker (at left) and Physical Science Technician Bernard L. Berger in Biochemistry Laboratory.

Figure 10:--Physiologist Jeannette R. Ernest at work on hematology of fish in the Physiology Laboratory.

Figure 11:--Vinyl plastic wading pools on levee. Pools are used for semi-field bioassays and for studies on the degradation of fish toxicants. Fishery Biologists Phillip S. Parker (left) and Philip A. Gilderhus observing bottom materials.

Figure 12:--An eighteen-foot, aluminum shocker boat rigged for 230-volt, DC power on trailer and parked in Holding House. Fishery Biologist Phillip S. Parker making adjustments.

Table 2:--Hematocrit values obtained with electronic and microhematocrit apparatus for seven species of fish.

Species	Number in sample	Hematocrit				Hemoglobin (gms./100cc.)	
		Electronic		Microhematocrit			
		mean	range	mean	range	mean	range
Rainbow trout	17	30.3	25.0-36.0	29.5	26.0-34.0	6.6	5.7- 8.0
Stoneroller	10	36.6	26.0-49.0	35.6	26.0-42.0	9.4	8.2-10.5
Goldfish	14	29.8	24.0-38.0	30.8	23.5-38.0	8.2	6.7-10.7
Golden shiner	22	36.6	29.0-44.0	--	--	8.5	7.0-10.0
Bluntnose minnow	22	39.0	24.5-61.0	37.3	24.5-55.0	10.0	5.5-15.0
Black bullhead	14	31.2	20.0-43.0	31.1	20.0-40.0	--	6.5-12.0
Largemouth bass	10	31.6	24.0-36.5	32.5	28.0-36.0	9.0	5.0-10.0
Largemouth bass	14	32.9	25.7-38.0	33.4	25.0-38.5	8.8	7.5-10.5

semi-field investigations on toxicants. Twenty-four vinyl plastic wading pools were erected and placed in service in the fall. They are being subjected to thorough testing to determine their usefulness as vessels for the bioassay of fish toxicants.

A portion of the hatchery garage space has been converted to a temporary laboratory. It has been equipped for analyses of water and fish. Thomas H. Lane took charge of the project in September.

STAFF

Dr. Robert E. Lennon, Chief
Mr. Charles R. Walker, Chemist
Mr. Phillip S. Parker, Fishery Biologist
Mr. Raymond E. Sampson, Hatchery Manager
Mr. Richard A. Schoettger, Fishery Biologist
Mr. Bernard L. Berger, Physical Science Technician
Miss Jeannette R. Ernest, Fishery Biologist
Mr. Thomas H. Lane, Fishery Biologist
Mr. Leif L. Marking, Chemist
Mr. Arnold M. Julin, Physical Science Technician
Mr. Rudolf E. Shawley, Fish Hatcheryman
Mrs. Delores A. Redmond, Clerk-Stenographer

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

An initial fish sampling program was completed and changes were developed for the 1963 season. Carp and carpsuckers were the most common large fish captured.

Monofilament nylon gill nets did not work satisfactorily for this sampling.

Electrical-shocker equipment was found to be the most suitable equipment for sampling shallow water habitat.

Age and growth analyses of carp showed that strong year classes were only produced during the first 3 years of impoundment. Growth rate of carp was more rapid in the Missouri River prior to impoundment than it is today.

FISH SAMPLING

A fish sampling program was initiated in the waters between Gavins Point Dam and Fort Randall Dam in May, 1962. This area includes Lewis and Clark Lake (30 miles long and 2 miles wide) and a 35-mile section of the Missouri River from the head of the reservoir to Fort Randall Dam. The waters were divided into areas and sub-areas and a regular sampling schedule was followed through the middle of October. The purposes of this sampling program were (1) to determine relative abundance of the various fish species; (2) to compare efficiency of different types of sampling gear in regard to habitat, sizes of fish, and season; and (3) to develop a sampling system that accurately monitors the fish population. Sampling gears used were gill net, frame net, electrical shocker, beach seine, and trawl.

A total of 83,548 fish (38 species) were captured. Exclusive of juvenile gizzard shad and minnows, the relative frequency of major species caught was as follows:

	Percent
Carp	30.2
River carpsucker	23.7
White crappie	8.0
Sauger	6.5
Freshwater drum	5.3
Channel catfish	4.4
Samllmouth buffalo	3.9
White bass	3.1
Shortnose gar	2.2
Shovelnose sturgeon	2.1
Others	10.6
	100.0

River carpsucker were taken in about equal numbers in both the river and reservoir, as were smallmouth buffalo. Carp, white crappie, sauger, freshwater drum, and channel catfish were taken almost entirely in the reservoir. White bass were taken only in the reservoir. Most shovelnose sturgeon and shortnose gar were taken in the river. Available evidence indicates that most species sampled in 1962 were collected in proportion to their relative abundance. One exception was the paddlefish which was too large to be captured by our gear.

Gill nets

Experimental monofilament nylon nets with two ranges of mesh sizes were fished to determine which were best suited for sampling reservoir fishes. Mesh sizes of one net were .5, 1.5, 2.5, 4.0, 6.0 inches and of the other were 1.0, 2.0, 3.0, 5.0, and 7.0 inches, stretch measure. Analysis of catch data indicated that a gill net with stretch meshes of 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 7.0 inches would have adequately sampled all sizes of fish captured by the two nets. A larger mesh gill net will be tried for paddlefish.

The monofilament nylon gill nets used were generally unsatisfactory because the filament in the smaller mesh sizes was easily torn while that of the larger meshes appeared to be too stiff for effective capture. As a result of these studies, a standard gill net for use during the 1963 field season will consist

of multifilament nylon of the following mesh sizes: 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 7.0 inches stretch measure.

Frame nets

Two sizes of frame net were fished during the 1962 field season. One net was constructed of 1.5-inch stretch mesh nylon webbing and the other of 2.5-inch webbing. Both nets were fished with 75-foot leads. Analysis of the catch of the major species by this gear indicated that, with one exception, the two nets did not differ significantly in size of fish captured. Small white crappie was captured in greater numbers by the smaller mesh; however, these fish were adequately sampled by other gear. The standard frame net adopted for use in 1963 will consist of 2.5-inch stretch mesh nylon webbing with a 75-foot lead.

Electrical shocker

A boom-type electric shocker was used for sampling shallow water areas. From May through early July, 220-volt alternating current was used. Thereafter, the same generator was converted to obtain 60-cycle pulsating direct current. It was obvious that direct current was much more effective for capture of young-of-the-year fish in Lewis and Clark Lake and appears to be our best method for sampling shallow areas.

Beach seine

Sizes and species of fish sampled by beach seine in 1962 were also taken by the boom-shocker. Shore seining in Lewis and Clark Lake is rather ineffective because of the absence of seinable areas, so regular sampling by this gear will be discontinued in the reservoir during 1963. Seining will be continued in the Missouri River between the head of the reservoir and Fort Randall tail-waters.

Trawl

In 1962 fishing was conducted from a 27-foot inboard boat using an 18-foot semi-balloon trawl. The reservoir contains many flooded tree areas and an accumulation of bottom debris, consequently few suitable trawling sites were located. Most common adult fish captured were carp, carpsucker, and smallmouth buffalo. Most common young-of-the-year fish were gizzard shad, white crappie, and drum. In 1963 a 28-foot semi-balloon trawl also will be used and trawling will be attempted with a smaller boat in the shallow water areas which could not be sampled with the 27-foot boat.

AGE AND GROWTH

Mounting and aging of fish scales collected during the 1962 field season were initiated in November. The large carp and carpsucker collections were sub-sampled, and mounting of scales has been completed. Scales from juvenile and some yearling fish are being mounted on glass slides; plastic impressions are being made from scales of older fish.

Aging of carp collected from Lewis and Clark Lake has been completed. The body-scale relationship was initially compiled by sex but no significant difference between sexes was found. The body-scale relationship is linear, and is described by the equation $Y = 0.634x - 13$. Start of scale formation was calculated to occur when the carp were 20.5 mm. long.

The average calculated total length of carp at each annulus is shown in table 1. From inspection of the table it appears that preimpoundment (reservoir closure, July 31, 1955) growth rate was more rapid than postimpoundment growth rate. Carp from Lewis and Clark Lake are smaller than carp taken from many other areas.

The age distribution of collected carp is shown in table 2. The 1955, 1956, and 1957 year classes predominate. These correspond to the year of dam closure and the two years following. Since 1957 reproduction or survival has apparently been poor. Our 1962 sampling data indicated a good 1962 year class.

Table 1:--Average calculated total length (mm.) of carp from Lewis and Clark Lake, 1962.

No.	Year Class	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
68	1961	85														
31	1960	91	142													
18	1959	99	137	170												
20	1958	97	150	185	212											
90	1957	110	160	193	217	234										
90	1956	94	165	203	232	251	267									
99	1955	81	184	224	250	279	290	307								
58	1954	86	197	243	281	300	335	355	372							
40	1953	79	197	246	277	311	336	362	384	401						
32	1952	63	171	233	264	323	359	389	416	436	449					
13	1951	79	167	242	281	322	363	414	440	460	476	488				
10	1950	108	160	222	296	334	394	426	454	474	493	508	522			
7	1949	112	170	264	306	349	388	437	470	493	520	538	552	563		
8	1948	103	166	243	305	348	385	425	468	506	538	555	570	582	595	
1	1947	138	186	221	260	325	427	453	518	561	581	594	608	672	633	642
Grand Aver. Cal. Length		90	171	217	249	278	313	352	403	439	479	519	548	576	599	642
Number of Fish		585	517	486	468	448	358	268	169	111	71	39	26	16	9	1

Table 2:--Age distribution of carp collected in Lewis and Clark Lake, 1962

Year Class	Age Group	Total No.	Percent
1961	I	173	4.9
1960	II	52	1.5
1959	III	29	.8
1958	IV	53	1.5
1957	V	741	21.0
1956	VI	1,025	29.0
1955	VII	810	23.0
1954	VIII	296	8.4
1953	IX	205	5.8
1952	X	69	2.0
1951	XI	30	.9
1950	XII	16	.5
1949	XIII	11	.3
1948	XIV	10	.3
1947	XV	1	--
Total		3,521	99.9

LIMNOLOGY

Analyses of the 1962 field data have not been completed. Efforts have been directed toward compiling past chemical and biological information collected on the Missouri River in this area to determine the changes in water quality associated with impoundment.

Water chemistry data have been collected at the Yankton city water plant from 1953 to the present. The impoundment of Fort Randall (70 miles upstream) in 1953 caused the alkalinity and hardness to decrease through 1955 and the seasonal fluctuations to be less extensive. Dr. Neel of the U. S. Public Health Service attributed the decrease in hardness to the fact that the weight of marl precipitation exceeded the weight of minerals leached from the bottom soils.

Since the impoundment of Lewis and Clark Lake (5 miles upstream) in 1957 there has been a gradual increase in water hardness. This increase can be attributed to the leaching of bottom soils. The high water exchange rate plus the shallow and exposed nature of Lewis and Clark Lake favors greater leaching than is possible in Fort Randall. Also, the Niobrara River which flows into the upper end of Lewis and Clark Lake carries new soils into the lake that are more available to leaching than the bottom soils in Fort Randall. There has been some evidence of increased photosynthetic

action in 1961 and 1962 over former years as shown by available weekly records of percentage saturation of dissolved oxygen, pH, and monocarbonate alkalinity. We plan to establish a monitoring system that will provide more adequate data on the alkalinity-oxygen complex.

Plankton counts at Yankton showed a relative green flagellate population four times greater than at any other water quality network station in the United States. The significance of this characteristic has not been determined; although organic enrichment is thought to encourage green flagellates, there is no evidence that pollution is a problem in Lewis and Clark Lake. The phytoplankton population is predominantly diatoms with Stephanodiscus as the dominant genus. Daphnia, Diaptomus, Cyclops and Leptodora are the most abundant macrocrustaceans.

SPECIAL PROJECTS

Richard Siefert is studying the early life history of the white crappie in Lewis and Clark Lake. His studies to date have included a literature summary, food of young-of-the-year crappie, and growth analysis. Copepoda (Diaptomus and Cyclops) were the most important food items but cladocera (Daphnia spp. and Leptodora) were common. David Vanicek is studying the growth rate, food, and condition factor of the sauger. A contract is being arranged with South Dakota State College for analyses of some fish stomachs collected during the 1962 field season. Three biologists are attending a class at the University of South Dakota on the use of IBM equipment for data analyses.

STAFF

Dr. Norman G. Benson, Chief
Mr. Charles H. Walburg, Fishery Biologist
Mr. William R. Nelson, Fishery Biologist
Mr. George A. Swanson, Fishery Biologist
Mr. Donald V. Swedberg, Fishery Biologist
Mr. Richard E. Siefert, Fishery Technician
Miss Harriet O. Eide, Clerk-Stenographer
Mr. Delbert H. Bridge, Maintenanceman

SOUTH CENTRAL RESERVOIR INVESTIGATIONS
Fayetteville, Arkansas
Thomas O. Duncan, Chief

A pre-impoundment study of the Beaver Reservoir area on the White River was initiated under contract with the University of Arkansas.

Preparations have been made for field studies of established White River reservoirs during 1963.

The General Services Administration leased space for office and laboratory facilities. We hope to occupy these quarters early in 1963.

GENERAL DESCRIPTION OF LOCATION

The reservoirs of primary concern to this investigation are located on the White River and its tributaries in northwest Arkansas and southwest Missouri in the Ozark Plateau. Existing reservoirs, with the exception of Lake Taneycomo, were constructed by the Corps of Engineers. Using the top of the power pool as base, the reservoirs total 138,750 acres. Individually the acreages are: Beaver (under construction), 28,220; Table Rock, 43,100; Bull Shoals, 45,440; and Norfork, 21,990. The existing reservoirs were constructed primarily for flood control and power, but also provide recreational benefits. Beaver Reservoir will also furnish a water supply for the large communities on the west side of the reservoir.

The White River is well known for its sport fishing. The important fish are large-mouth, smallmouth, and spotted bass, crappie, catfishes, and walleye. The reservoir tail-waters were found suitable for trout and rain-bow trout are now stocked. Brown trout were planted in the tailwaters of Norfork and Bull Shoals. Lake Taneycomo, formed by a low dam and operated by a private power company, is considered a cold water lake, more or less part of the Table Rock Reservoir tailwater; it is stocked with rainbow trout by the State of Missouri. Rainbow trout have been experi-mentally stocked in Bull Shoals Reservoir, and are occasionally taken by anglers.

INVESTIGATIONS OF EXISTING RESERVOIRS

Work in 1962 has been largely limited to preparatory field reconnaissance and litera-ture review. The plans for 1963 field operations call for general surveys, testing equipment and techniques. A series of spot poisonings, echo-soundings, and water chemistry data will be taken at various stations which will include locations in open water areas, shore areas, and small coves. An attempt will be made to assess the value of spot poisonings in relation to an echogram transect of the area sampled. Also, information will be obtained on various aspects of life histories of the several fish species in the reservoirs. Collections are planned for bottom fauna and plankton. Experi-mental midwater trawling will be tested as a sampling method. Staffing and the procurement of equipment have been deferred due to the lack of storage, office, and laboratory space. Presently, the staff consists of two fishery biologists (research) and one clerk-typist. Five additional fishery biologist (research) positions are in the stages of being established or remain vacant, pending the new space.

The University of Arkansas generously assisted the investigations by providing a temporary office since April 1962. During December, a lease was finally negotiated by GSA for 1,778 square feet of space in downtown Fayetteville and the leasor immediately started required alterations.

PRE-IMPOUNDMENT STUDY

A two-year contract was negotiated with the University of Arkansas in October 1961 for pre-impoundment study of the Beaver Reservoir area. Units included in the contract were in the fields of agronomy (both soils and water), botany, bacteriology, fisheries, and fish parasitology. An amendment was made to the contract in July 1962 to provide for investigation of the aquatic insect fauna of the area to be impounded. In addition, the Arkansas

Game and Fish Commission sponsored research on the zooplankton of the same area. Several of the studies will be the first compilations for this particular watershed, i.e., fish parasites, aquatic insects, plankton, and algae.

Brief summaries of each of the contract units follow:

Soils:--Sampling sites were selected with the aid of aerial photographs. A preliminary general survey of the soils in Beaver Reservoir area was completed and summarized in a general soils map, using a legend modified from current Soil Conservation Service General Soils Maps. Water sampling and analysis were started during the last quarter of the year. Water samples are being analyzed in the field by a colorimetric system.

Botany:--A vegetation analysis for 30 different stands of forest in the reservoir basin is in preparation. Tree data included information on the species present, size, basal area, density, and frequency (distribution). This will be converted to a per-acre basis. A total of 1,050 vegetation samples was taken from the various sites using the augmented Bitterlick method. Shrubs, vines, small tree seedlings, and herbaceous species of the forest floor were sampled by use of quadrats, the point frame, and a modification of the ocular-tube method developed by project personnel. The data include species present, cover, density, and weight of understory vegetation. These data are being converted to absolute and relative values per acre. Sampling included 575 foot-square plots to determine species present, density and weight, and approximately 1,850 ground cover estimates with the ocular tube. In addition, the weight of mulch on the forest floor was determined from randomly distributed foot-square quadrats. Much of this information has been compiled.

Sampling sites and plots were located on overlays of large scale maps (6 inches to the mile). Over 1,500 plant specimens were collected as voucher specimens or for identification purposes.

Microbiology:--Sampling techniques for this research were established. Wood samples were collected from dominent tree species in the basin, allowed to dry, cut to uniform size, and immersed in river water. Every 30 days, the samples are tested for changes in pH, turbidity, and color. Algae and fungi are being collected and identified, and it is planned that a complete list of algae will be made for the Beaver Reservoir area. All collecting sites were tested for sewage pollution every two weeks beginning in August. Methods were developed and tested for determining the nannoplankton of the basin drainage. Culture techniques for microscopic algae have been developed, and were utilized to isolate in pure culture a blue-green alga causing a bloom in Brush Creek.

Fisheries:--An electric shocker was constructed for inventory collections of fishes. In addition, straight and bag seines were used to supplement and compare selectivities of the shocker catch. In the laboratory, each collection was separated to species and enumerated.

Parasitology:--Specimens of the several "basses" were obtained from the collection sites on the White River or its tributaries within the Beaver Reservoir site. The species of fish and the number of each examined for parasites were: Micropterus punctulatus 162; M. salmoides 33; M. dolomieui 18; and Roccus chrysops 1. In R. chrysops only cestodes were found. Acanthocephala, nematodes, copepods, and cestodes were found in Micropterus species. Leeches were found in all Micropterus species except M. salmoides. The parasites were preserved in vials of 70 percent ethyl alcohol and numerous specimens were stained and mounted on slides for identification purposes.

Entomology:--Prior to the arrival of a graduate student in late November, some preliminary collecting was accomplished on this study. Subsequently, equipment was ordered, literature searched, and some collecting undertaken.

S T A F F

Mr. Thomas O. Duncan, Chief
Mr. James W. Mullan, Fishery Biologist
Mrs. Octavia R. Vanderslice, Clerk-typist

SANDY HOOK MARINE LABORATORY
Highlands, New Jersey
L. A. Walford, Chief

HIGHLIGHTS

Publication of a complete Sportsmen's Guide to Sharks of the Northeast United States and the rapidly increasing interest by sportsmen in shark fishing.

Discovery of the large variety and great abundance of sharks in shallow middle Atlantic waters, and compilation of many new occurrence records of sharks.

Completion of analysis of the salt water angling survey for 1960.

Compiling and editing of a volume of 25 contributions on sea water supply systems.

Inception of monthly surveys of surface temperatures of the Continental Shelf, Block Island to Cape Henlopen, in cooperation with the U. S. Coast Guard; data for environmental study and distribution to sportsmen.

Establishment of working methods for the culture of microorganisms, including techniques for physiological study for routine vitamin B_{12} bioassay of marine waters.

Development of analytical techniques to relate occurrence of migratory fishes to environmental factors.

Development of techniques for controlled testing of the toxicity of marine pollutants, including pesticides, detergents and fallout products; actual and test runs with many marine species.

REVIEW

The Atlantic marine game fish program is dedicated to the goal of understanding the mechanisms controlling distribution, migratory habits and changes in abundance of species. Because distributions extend over hundreds or sometimes thousands of miles, our goal can be approached only by studying species and their environments over their entire ranges. To do this will require large scale continuous and simultaneous field observations, and will be possible only by the collaboration of all marine laboratories sharing the same interest; and by the help of volunteer observers, including game fishermen and diver-naturalists. The program of the Sandy Hook Laboratory so far has been oriented towards fostering this collaboration and assistance. The laboratory program at Sandy Hook has concentrated on (1) assembling and analyzing existing data pertaining to coastal game fishes to develop bases for designing large scale studies of species; (2) developing methods of bioassay using phytoplankters for measuring biological productivity in estuaries and coastal waters; (3) measuring the effects of chemical wastes on marine life.

Lack of vessel facilities has limited our field program. Nevertheless, we have been able to conduct a study of sharks during the late summer and fall to determine species composition, sizes, food and relative abundance and to encourage sport fishing for these interesting though predaceous animals. Graduate students at the University of Miami working under appointments given by the Sandy Hook Laboratory, have worked on phases of the biology of the red drum and the bluefish.

LIFE HISTORY STUDIES

Red drum

Mr. Bernard Yokel completed the field work for a study of red drum (Sciaenops ocellata) which he conducted while in the employ of the laboratory. The data are now being analyzed and, when completed, will be submitted as a master's thesis at the University of Miami.

The project included (1) a study of food habits in which the stomachs of over 600 red drum were examined; (2) observations of feeding behaviour of specimens maintained captivity in

the Miami Seaquarium; (3) a study of the biogeography of the species, from literature sources, direct observation and field interviews and (4) a study of age and growth involving some 1,000 specimens.

Bluefish

Herbert G. Anderson, Jr., commenced a study of macroscopic parasites of 129 bluefish (Pomatomus saltatrix). Samples were collected during the summer from three regions representing the north, central, and southeast coast of the United States. The parasites identified consisted of: Copepoda (2 species), Acanthocephala (2), Trematoda (3), Nematoda (2), Isopoda (1), and Cestoda (2).

The percentage incidence and the average rate of infection of each parasite was calculated for 67 fish from Sandy Hook, New Jersey, 35 fish from Hatteras, North Carolina, and 27 fish from Miami, Florida. These data were analyzed to show variation due to size and sex. The percentage incidence of gill parasites was analyzed to show interrelationships. A winter sample of 100 bluefish has been collected from southern Florida.

Sharks

Interest in sport fishing for sharks is on the rise. Over 75 sportsmen and sportsmen's clubs have formally registered as cooperators, and have agreed to keep records, participate in the tagging program, or turn over to the laboratory their shark catches. Some have begun participation already; others will receive the standard log forms and tagging materials for the coming season.

Tagging:--On a cooperative cruise with the Woods Hole Oceanographic Institution to Hydrographer and Veatch Canyons, various dart tags were tested on sharks. Thirty-eight sharks (33 blues, 4 makos, and 1 hammerhead) were marked with plastic spaghetti tags attached to stainless steel darts. Each tag was attached to an adapter on a ten foot pole and inserted into the back of the shark near the first dorsal fin. Sportsmen will be provided with tags of this type for the more extensive tagging operations planned for 1963.

Longlining:--From July 1 to August 22, John G. Casey conducted longline fishing from skiffs and sport fishing boats along nearby beaches and in Sandy Hook and Delaware Bays. One trip to Delaware Bay was a joint cruise with the University of Delaware Bayside Marine Laboratory aboard their research vessel Wolverine. These efforts were directed to surveying sharks in the shallow inshore zone.

In July, the laboratory acquired the 65-foot research boat, Challenger, which was outfitted for longline fishing, and from August 23 to October 11, sixteen cruises in nearby coastal waters were made. During these cruises, about 100-150 hooks were set at each fishing station. The gear was allowed to fish for about two hours and then was retrieved by hand. At each station, sea and weather conditions were recorded as well as surface to bottom temperatures and salinities.

Species represented in the total catch of 133 sharks from 33 inshore longline stations were the sandbar, dusky, tiger, sand, hammerhead, great white, bull, bignose, and smooth dogfish. Data were obtained from the offshore tagging cruise and catches of sport and commercial fishermen on 85 additional sharks including makos, blues, and bigeye thresher.

The shallow waters of Delaware Bay were found to be a principal pupping ground for the sandbar shark (Carcharhinus milberti). Catches of this species (pups, 20-24 inches, juveniles, 3-4 feet and adults 6-7 feet) in Delaware Bay during August suggest that their distribution is influenced by depth. Pups were taken in inshore areas (3-15 feet), juveniles in intermediate depths (20-30 feet), and adults in the deeper areas only (70 feet). Sandy Hook and Raritan Bays are not important pupping grounds.

Sharks are more abundant in Delaware Bay than in Sandy Hook Bay. Five species were landed in Delaware Bay -- sand, bull, dusky, sandbar, and smooth dogfish. These were taken at four locations. Large hammerhead, tiger, and great white sharks frequent the inshore shallow waters bordering Sandy Hook (less than one mile). The most common large hammerhead in this area was found to be the

southern hammerhead, Sphyrna lewini, contrary to popular literature. Sandbar and dusky sharks were the most common species in the longline catches of both 1961 and 1962. Shark catches decreased abruptly in late September and none, except dogfish, was landed after October 11.

Examination of sharks included 20 morphometric measurements, examining stomachs and gonads, and collecting samples of fins, jaws, denticles, skulls, vertebral columns and parasites. Thirty-three food items, including garbage and sport fishing hooks, were found in the stomachs of 93 sharks. Bottom fishes (headfish, sea robins, flat fishes, skates) were the most common food item. Pelagic fishes eaten included menhaden, bluefish, scup, mackerel, and squid.

ENVIRONMENTAL STUDIES

Environmental analysis

Study of all available data for 14 species of Atlantic and Gulf Coast game fishes has brought out patterns of distribution in space and time which have remained consistent over the period 1958-1961. These patterns provide the bases of hypotheses for the design of life history studies, indicating a rational scheduling of field operations such as sampling and tagging.

There is considerable variation in the ranges of the species studied and in the seasons when they are most available to coastal fishermen. Fishes which overlap geographically tend to favor the same areas, even though they may occur at different times. It appears then that some stretches of coast are regularly much better fishing grounds than others.

Evidently this results partly from geographic differences in the temperature regimen and partly from characteristics of bottom topography and sediments. No doubt other factors of environment influence distribution, but there can be no way of taking these into account until data are collected simultaneously and systematically all along the Atlantic and Gulf coasts.

It is evident from our analysis of existing data that the movements of fish populations cannot be explained wholly by extrinsic factors. Something within the fish -- a biological clock mechanism whose precise timing seems to be associated with the sun's angle of declination -- impels them to migrate at certain times north and south to their summer and winter destinations.

Pollution

Acute toxicity of an organophosphorous insecticide (parathion), a chlorinated hydrocarbon insecticide (p,p'DDT), a soap, and a detergent to six species of juvenile marine fishes was determined. The results are summarized in the table at the top of the following page.

In all cases, small fishes were more susceptible than large ones of the same species. The detergent remained toxic even after 5 weeks in solution.

Salinity of the ambient medium affects the toxicity of test compounds, but the pattern appears different for each compound. Preliminary experiments with F. heteroclitus show that parathion becomes progressively more toxic with increasing salinity over 20 °/oo 1/; that DDT is most toxic at estuarine salinities (15 - 25 °/oo) and rapidly loses lethal effects below 13 °/oo and above 29 °/oo; that soap is most harmful in waters of low salinity; and that detergent, like parathion, increases in toxicity with increasing salinity over 21 °/oo.

Some experiments in progress include chronic exposure of juvenile marine fishes to sub-lethal concentrations of detergent and to DDT. Also in progress are interaction effect of insecticides and detergents at sub-lethal levels. Preliminary observations after 6 weeks

1/ Preliminary experiments with juvenile eels suggest that parathion is far more toxic at 5 °/oo than at 20 °/oo salinity; however, the two species of fish are sufficiently unlike that a comparison is not justifiable.

Species	Approximate size (mm)	Dose, in ppm, which will kill 50% of the test fish in 96 hours*			
		p,p'DDT**	parathion**	soap***	detergent***
sticklebacks (Apeltes quadracus)	32	0.0049	****	****	****
mullet (Mugil cephalus)	45	0.0009	4.4	580.	10.1
winter flounder (Pseudopleuronectes americanus)	55	0.0039	****	****	8.2
eel (Anguilla rostrata)	60	0.0041	4.8	****	****
mummichog (Fundulus heteroclitus)	38	0.0032	5.2	1400.	22.5
silverside (Menidia menidia)	59	0.0004	2.0	790.	7.0

* Test conditions: The animals were procured from Sandy Hook Bay and all were actively feeding for two weeks prior to study. Test containers were 20-liter glass jars filled with 19 liters of filtered sea water of salinity 23 ppt. and pH 7.6. Jars covered with glass covers, supplied with aeration via glass tubing. Temperature 20° C. No food was offered during exposure. Dead fish were removed daily. Minimum of six dosages.

** Active ingredients

*** Packaged product

**** Experiment not conducted

---

of chronic exposure to detergent indicate that dosages of 5, 8, and 10 ppm are harmful and often fatal to young Fundulus, with survival much better at low (i.e. 10 °/oo) salinities.

Dose (ppm)	Gonadosomatic index (Ovary weight/body weight x 100)
0	1.9
10	1.6
100	1.3

Chemosterilant research

Compounds which sterilize mosquitos and other insects have received much attention. Ronald Eisler screened apholate (an experimental chemosterilant, Olin-Mathieson Co.) on fish (Fundulus majalis and F. heteroclitus), shrimp (Palaemonetes vulgaris), and snails (Nassa obsoleta). Short term exposure of higher animals to dosages of 5,000 ppm produced no mortality by 96 hours; however, long term exposure to dosages higher than 100 ppm resulted in heavy mortality. Ovaries of female F. majalis sacrificed on the 19th day post-exposure were noticeably affected:

Continental shelf hydrography

Monthly surveys of surface temperatures of coastal waters from Block Island to Cape Henlopen were commenced by John Clark in co-operation with the U. S. Coast Guard Air Base at Floyd Bennet Field, Brooklyn. The work is the laboratory's contribution to an expanded coast wide oceanographic effort, the Atlantic Shelf Campaign, which is to reach full emphasis in 1965 or 1966.

Present plans are for a monthly flight along a 1,200-mile flight track at an altitude of 500 feet, as shown on the flight plan (see Isotherm chart p. 105). Five drift bottles and five sea bed drifters are dropped at 10-mile intervals along the flight track (these are furnished by the Woods Hole Ocenaographic Institution).

The Aerial Shelf Survey is designed to provide, at intervals, near-simultaneous surface temperatures for the continental shelf of the New York Bight. Also, surface and bottom currents are studied through use of drift bottles and sea bed drifters.

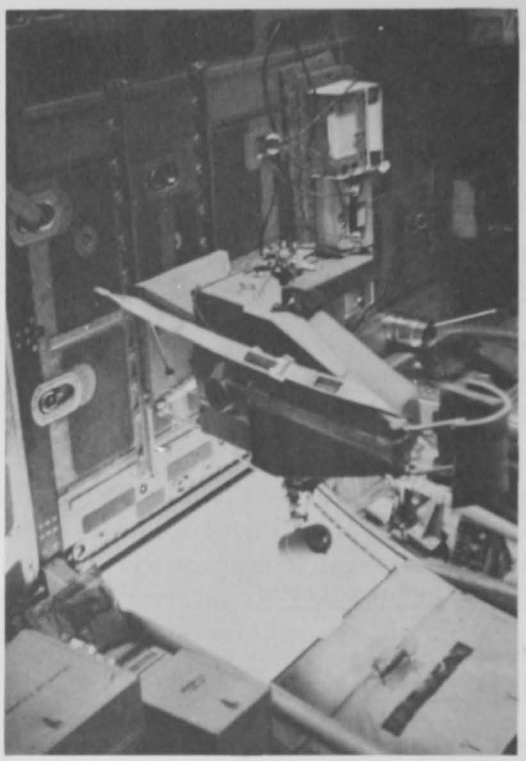
The survey results will be useful not only for predicting the occurrence of fishes, but also in providing data for physical oceanographic and meterological research. Studies of the relation of surface temperature to general oceanographic phenomena in progress at various laboratories give encouragement that knowledge of subsurface temperatures and currents can be deduced from surface observations.

The aerial survey approach is economically feasible because of (1) the cooperation of the U. S. Coast Guard in furnishing an aircraft (Grumman "Albatross") and crew for the flights, and (2) the recent availability of inexpensive (less than $2,000) production models of the infra-red radiation thermometer (Barnes Engineering Corp., Stamford, Conn.). The I.R.T. measures the emission of infra-red (8-13 band) from the sea surface by converting it to an electrical impulse which, in turn, is read out on a meter calibrated to show temperature in °F.

Temperatures are monitored continuously along the flight path on a strip chart recorder (see Isotherm chart on p. 105). Frequent in-flight checks are made against a known temperature source in the plane and against surface light ship temperature records (Ambrose, Barnegat, and Delaware Light vessels).

Assay techniques

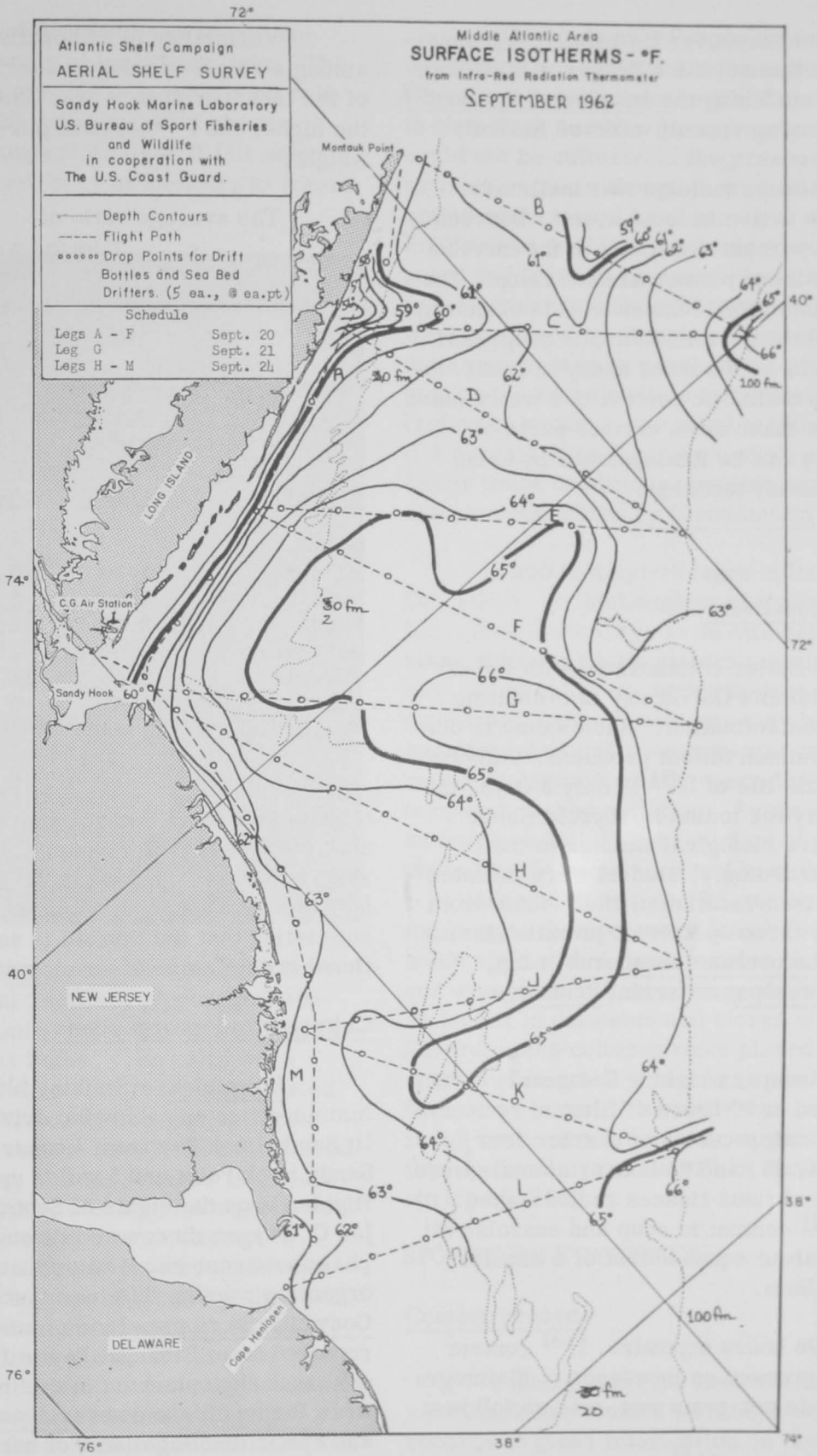
Jan C. Prager has been developing assay procedures for charting the productivity of



Infrared thermometer (IRT) positioned in Grumman "Albatross" for making sea surface temperature surveys of waters along the Continental Shelf from Block Island to Cape Henlopen.

coastal water masses. There is need for a precise microbiological assay procedure applicable to both vitamins and "fuel" nutrients that can be done rapidly (3-4 days), on shipboard, and using a minimum of laboratory facilities.

Gyrodinium fissum seemed a logical organism for this assay as it required organic nutrients and reproduced rapidly (population growth is the usual end point in microbiological assay). Early work with Gyrodinium fissum was abandoned, however, in favor of Platymonas subcordiformis, a fast growing marine chrysomonad. Nutritional studies on Platymonas indicate that it requires vitamin B_{12}, but is not particularly sensitive to concentration gradients greater than 1×10^{-9} mg. percent. Both biotin and thiamine are necessary for its growth. Platymonas has a high osmotic tolerance and, when given nutrients in concentrations approaching upper tolerance levels, will produce 8-900,000 cells/ml. in 4-5 days of incubation. Thus Platymonas met the criteria of a good



Isotherm chart indicating flight path along Continental Shelf and isotherm pattern emerging from translation of temperature records to chart.

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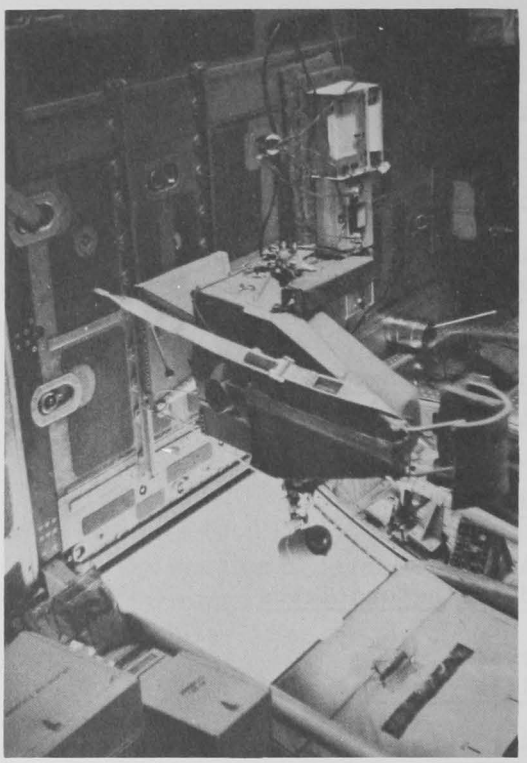

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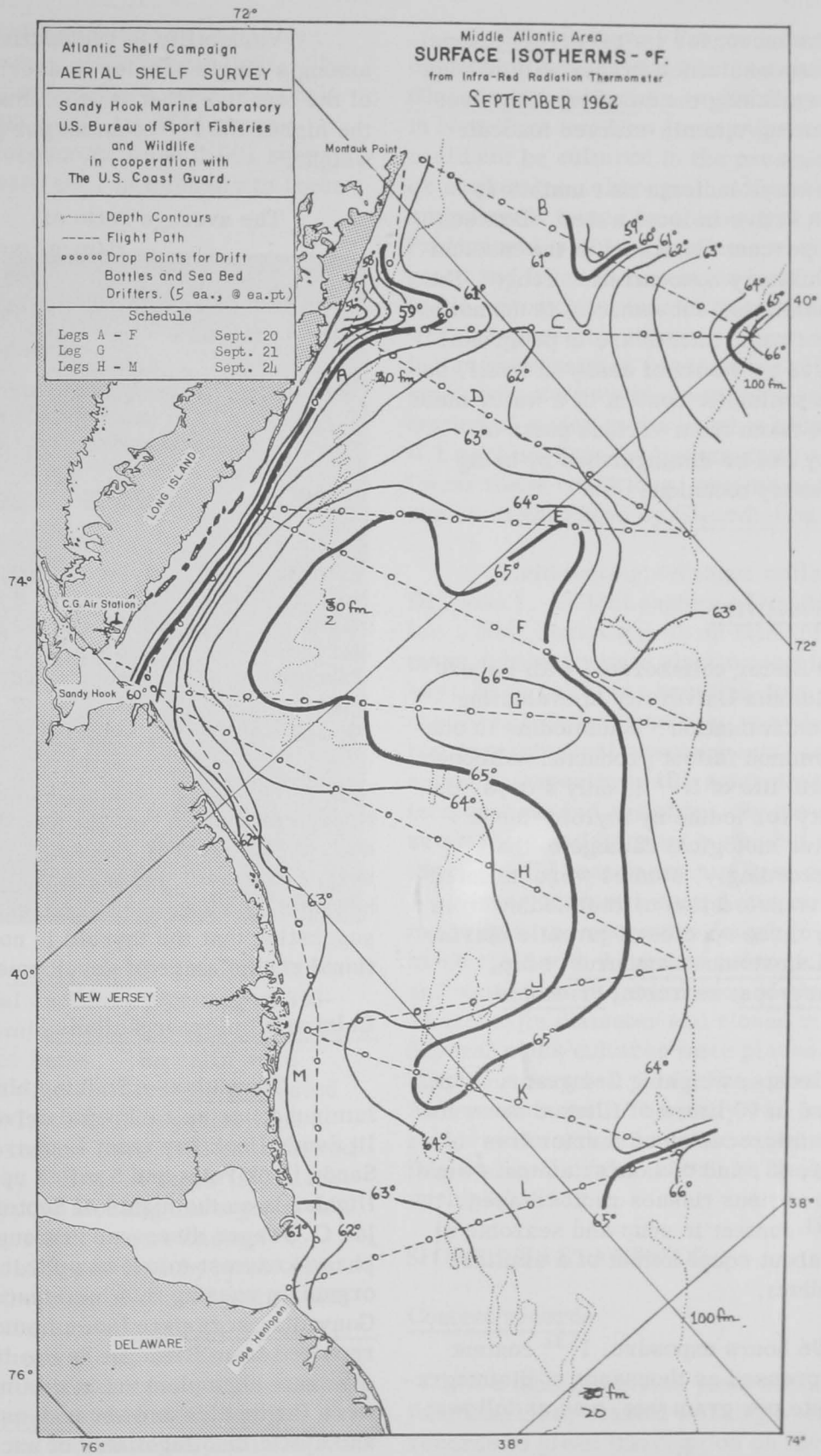


Isotherm chart indicating flight path along Continental Shelf and isotherm pattern emerging from translation of temperature records to chart.

assay organism except for its relatively insensitive vitamin response which Prager will attempt to sharpen by enriching the basal medium used for assay and using vitamin-starved innocula.

Trial assays indicate that inhibitory substances are active in local water. Between 0.5 - 1.5 ml. percent sea water in the medium is the most inhibitory concentration range. The nature of the inhibiting substance(s) is unknown, but replacement experiments are in progress. Inhibition serves as a sort of assay in itself, as an index of the antibiotic content of a water mass. Water samples taken from various parts of Sandy Hook Bay can be distinguished by using the inhibition assay technique.

PHYSIOLOGY

Effects of radioactivity

Ronald Eisler collaborated with Robert Kirchen of Columbia University in evaluating aquatic radiocontamination. Radioiodine is one of the more common fallout products. Although the physical half-life of I^{131} is only 8 days, the peculiar affinity for iodine by thyroid tissue causes extensive biological damage to that structure. Accordingly, studies were initiated on uptake and translocation of radioiodine from the medium by three species of juvenile marine fishes (spot, Leiostomus xanthurus; scup, Stenotomus chrysops; searobin, Prionotus evolans).

The teleosts, weighing 2-4 grams each, were immersed in 10 liters of filtered sea water containing 100 microcuries of carrier free NaI^{131}. At 24, 48, and 96 hours, animals were sacrificed and various tissues radioassayed. Total body I^{131} content in scup and searobin at 96 hours was about equal to that of a similar volume of medium.

After 96 hours exposure, I^{131} content in muscle, expressed as thousands of disintegrations per minute per gram wet, was as follows:

Species	Average I^{131} Activity	Range
Searobin	55.0	50 - 60
Scup	39.5	30 - 57
Spot	6.0	4 - 8

Variability in radioactive iodine content among a single species is ascribed to the size of the test fish; that is, the smaller the fish, the higher the I^{131} content per gram body weight.

The average ratio of

$$\frac{\text{d/m/g/ wet various tissues}}{\text{d/m/g wet muscle}}$$

for each species after 96 hours exposure is shown in table 2.

Table 2:--Average ratio of disintegrations/min/gram of certain tissues/muscle (wet).

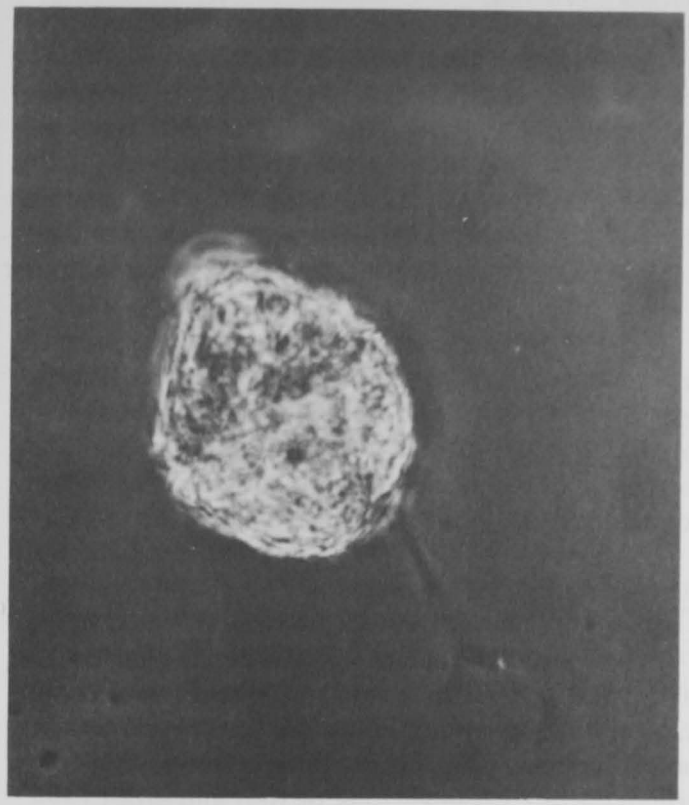
Tissue	Searobin	Scup	Spot
Gill	2.89	5.83	20.79
Viscera	1.80	7.75	22.14
Muscle	1.00	1.00	1.00
Skin	2.55	2.44	2.49
Vertebrae	0.67	1.15	1.63
Thyroid	2.66	18.19	58.88
Heart	1.81	3.56	3.33
Whole Fish	1.34	2.43	5.00

Table 2 indicates that iodine-seeking tissues in spot and scup, in order of specific activity, are thyroid, viscera, gill, heart, skin, vertebrae, and muscle. In searobin, however, variation among tissues is small, suggesting that the thyroid is not fully functional at this stage of development.

Culture of microorganisms

Gonyaulax:--Striking blue-white luminescence on each wind driven wave crest lit Sandy Hook Bay from Leonardo across to Sandy Hook Point and 5 miles up the bay to Highlands on the nights of September 16 - 18. Jan C. Prager discovered through anoptral phase-contrast microscopy that the dominant organism causing this luminescence was Gonyaulax scrippsae (not common here, but reported from Barnegat Bay in 1929). Subdominant phytoplankton in this massive bloom were Euglenoids and several young trophic and cystic dinoflagellates of uncertain identity. The water glowed blue uniformly rather than flashing in distinct spots, evidence of microbially produced luminescence. Gonyaulax scrippsae was not known to luminesce, although

several members of its genus are prime research tools in studies of biological light. The following photomicrograph of a swimming cell was taken at 1,280X using anoptral phase-contrast and a stroboscopic flash 0.001 second in duration, 150 watt-sec. in intensity to freeze the motion.



Gonyaulax scrippsae was isolated from the bloom by serial capillary pipetting of 300-500 actively swimming cells through 12 sterile artificial sea water baths. The cells were established in test tube cultures and incubated at 18±1° C. in 500 ft-c. of fluorescent light. These bacterized strains have been subcultured five times thus far, and Mr. Prager is collaborating with Dr. J. J. A. McLaughlin's group at Haskin Laboratories Inc., in an attempt to make the cultures bacteria-free by antibiotic treatment. With the hope of understanding the natural causes of the luminescent bloom in Sandy Hook Bay, these axenic cultures will be used to determine the nutritional requirements of Gonyaulax scrippsae and to study in vitro requirements for its sudden rapid proliferation. The chemical nature of synthetic sea water in which it grows gives some insight into its needs, and these seem to correlate with hydrographic conditions in the bay at the time of the luminescent bloom.

Glenodinium:--Prager was successful in developing a technique for the culture of Glenodinium foliaceum, a dinoflagellate common in New York and New Jersey waters. G. foliaceum could not be cultured in the presence of proline or methionine, although it utilized all other amino acids tested as a source of nitrogen. Proline and methionine effects were investigated and found to be caused by drastic changes in pH. Stock solutions of these amino acids were prepared by dissolving them in HCl, and it was necessary to readjust the pH of each stock solution as well as to increase the concentration of buffer in the basal medium (0.05 to 0.1 percent Tris (hydroxymethyl) aminomethane). Under these conditions, proline and methionine can be metabolized by Glenodinium foliaceum.

Field testing:--Prager collaborated with Dr. John J. A. McLaughlin of Haskin Laboratories, Inc., New York City, in an attempt to grow axenic mass cultures within plastic containers in the bay. Such cultures, bacteria-free and in chemically defined medium, may be utilized to compare physical environmental conditions in nature to experimentally controlled light, temperature and agitation. By the end of the summer, a satisfactory method of anchoring these fragile containers had been developed. A cylinder of plastic coated garden fencing made up to the necessary length (5-10 feet) was made rigid with 4 bamboo poles tied lengthwise, and was given an inner lining of plastic tubing 24 inches in diameter and closed at one end. Several mass cultures were placed inside the apparatus in small three-liter plastic containers with screw caps. The study was stopped by rough water which made the apparatus inaccessible by small craft. It will be resumed in the spring of 1963.

SPORT FISH STATISTICS

Contest records

Fishing contests yield useful data and John Clark has started working over contest records to glean information on the distribution of fishes. Clark's first effort in this direction was an analysis of bluefish catches reported in the Schaefer Contest of 1961 (courtesy of Peter Fitzpatrick of the F & M Schaefer Brewing Corp.).

The available data showed a regular progression in weight of the contest blues from 4-1/2 pounds in June to over 6 pounds in October, in the middle Atlantic and northeast coasts. Peak fishing came in early August and again in late September -- the first peak for New York and New Jersey, the second for New England. Bluefish were taken in good numbers around the clock, but the largest catches and the largest fish were taken in two of 6 4-hour time periods: 4-8 a.m. and 8 a.m. - noon. The smallest contest fish were taken in Rhode Island and Connecticut with larger fish taken both north and south.

Striped bass were taken in quantity from mid-June to mid-November, with contest fish averaging 27 pounds. Peak catch was in early October. Fish were taken in greatest abundance in time periods 12-4 a.m. in the northeast and 4 a.m. - noon in the middle Atlantic.

National survey

The analysis of salt-water angling for 1960 was completed and published. Estimates place the total weight of the 633 million fishes taken in 1960 at 1.4 billion pounds. The sport catch makes up about 40 percent of the combined commercial and sport catch of edible fin fish. The most polular fishes (in numbers) were sea trouts (83,800,000) and flatfishes (50,600,000). Most people fished from boats.

Current statistics:--Data from poll-type surveys are of value, but no substitute for detailed statistics collected on a current basis. Only by obtaining regular catch data directly from fishermen all along our coasts can problem areas be pin-pointed for research. As a start in this direction, John Calrk has commenced a trial program of volunteer reporting - twenty anglers keep daily logs of their fishing activities including data on gear, bait, tides, winds, sea state, etc., as well as fish data.

FAUNAL SURVEY

Tidal area survey

Mr. Jonathan Baskin was employed to direct a survey of tidal nursery areas in estuary, bay, and ocean environments in the Sandy Hook area. Ten stations were sampled by seine at weekly intervals from late June to early September. Special hauls were also made to check the influence of tide stage and time of day.

Although this is, for the most part, a high salinity system and thus not typical of the massive estuaries to the south, it was found to be an important nursery ground for bluefish and other angling favorites such as flounders, as well as for important forage fishes like spearing. This sampling program was conducted by 10 of a crew of 18 student-apprentices who worked and studied in the laboratory this summer.

These data are the subject of a report by a Rutgers graduate student, David Williams, entitled "An Application of Linear Regression Analysis to a Fish Survey". The complex analysis of the effect of environmental variation on occurrence of the 33 species is proceeding.

Underwater

Our program has continued to benefit from the assistance of the American Littoral Society, a national society of skin divers who are also naturalists (usually amateur) and who record their observations of fish and other fauna while diving. Records of occurrence have been listed for greater amberjack, fluke, snowy grouper, common jack, a crab, Lopholithodes, and pumpkin seed. Behaviour patterns have been recorded for bluefish, menhaden, blackfish, and others.

The society has initiated a publication, the Underwater Naturalist, which serves not only to inform members, but also as a place for them to record their observations, namely, a section of Reports and another of Field Notes. This is a first class letterpress magazine which fulfills an important role and, from appearances, may distinguish itself in the conservation and natural history field. The laboratory provides guidance for the society and its publication.

Members of American Littoral Society participating in annual fish count, noting various species on charts.

OTHER COOPERATIVE RESEARCH

Office of Naval Research Fellowships

The following researchers were supported by the Office of Naval Research during the course of their studies here: Dr. Kawamura of Hokkaido University, who departed on September 20 after completing a three-month study of the distribution of phytoplankton in the Shrewsbury estuary system; Dr. Yamazi, University of Kyoto, who departed the laboratory after completing a study of zooplankton distribution similar in nature to that conducted by Dr. Kawamura; Dr. Della Croce, who departed for Italy in December after completing his study of the cladoceran Penilia, involving factors affecting annual cycles and occurrence.

National Science Foundation shark study

Dr. Sheldon Applegate of Duke University worked closely with John G. Casey throughout the summer, collecting specimens from species which related to his studies of the evolution of sharks, particularly Carcharias taurus, the sand shark.

Rutgers University projects

Michael LaMarca, Rutgers University, collected blood and tissue samples from tiger and great white sharks. Dr. Westman, with two of his students, studied the effects of contaminating agents on the market acceptability of fluke.

AWARDS AND HONORS

Student trainee Joan Bradley won second place honors in the Central Jersey Science Fair at Rutgers University with her study of comparative salinity toleration ranges of three species of marine algae maintained in axenic culture and chemically defined medium. Student trainee Sidney Lekach won third place honors at the same fair and first place honors at Rutgers University College of Agriculture Science Fair for his work on a closed ecological system which supports a small mammal utilizing a marine alga for oxygen, food, and waste removal.

Hartwick College, Oneonta, New York, conferred an honorary degree of Doctor of Philosophy upon Lionel A. Walford at commencement exercises in June.

S T A F F

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Mr. John R. Clark, Fishery Biologist
Dr. Ronald Eisler, Fishery Biologist
Dr. Jan C. Prager, Fishery Biologist
Mr. John G. Casey, Fishery Biologist
Mr. William P. Jensen, Administrative Assistant
Mrs. Theodora R. Branch, Librarian
Mr. David G. Deuel, Fishery Biologist
Miss Grace K. Donahue, Secretary
Mrs. Roberta M. Carter, Illustrator
Mr. John B. Mahoney, Fishery Technician
Mr. Robert I. Wicklund, Fishery Aid
Miss June I. Krayl, Clerk-Typist
Mr. Lewis G. Maxie, Maintenanceman
Mr. Solomon Adams, Laborer

TIBURON MARINE LABORATORY
Tiburon, California
Gerald B. Talbot, Chief

The Tiburon Marine Laboratory is located at the Navy's deactivated submarine net depot on the Tiburon Peninsula north of San Francisco on the west side of San Francisco Bay. Two buildings are occupied under license from the Navy. These have been partially rehabilitated for office use, and space has been provided on a temporary basis to the Bureau of Commercial Fisheries as headquarters for its Pacific Coast shellfish research.

The net depot has been deactivated for almost 5 years, and there is a possibility that it may be declared surplus to the Navy's needs. Many government and private agencies and institutions have requested it for their use, including the Department of the Interior, and continued use of the presently occupied buildings is not assured. Consequently, we have delayed the installation of laboratory facilities and sea water system in the buildings now occupied until some decision is made. Most projects undertaken have, therefore, been carried out in the field.

An "Atlas of Pacific Marine Game Fishing" was completed and submitted for publication. The atlas consists of 21 charts showing the major fishing areas, a list of the more important species taken in each area, and indications of sport fishing facilities available, such as launching ramps, sport fishing or charter boats, and rental skiffs. This graphic presentation of marine game fishing for the west coast, Alaska, and Hawaii is overlayed on conventional U. S. Geological Survey and Coast and Geodetic base charts and provides a detailed and accurate description of important game fishing areas. In addition to an introductory text, a list of about 250 common species of the west coast and Hawaii is included in the atlas.

In cooperation with the U. S. Navy, personnel of the Tiburon Marine Laboratory dropped a total of 125 plastic sea bed drifters at 25 stations in the Monterey Bay area. This is the first time that sea bed drifters have been used on the Pacific Coast, and the first time that an aircraft has been used to distribute the oceanographic devices at a series of stations. The drop was completed in one hour, in comparison to an estimated time of 15 hours for a boat to accomplish the same task.

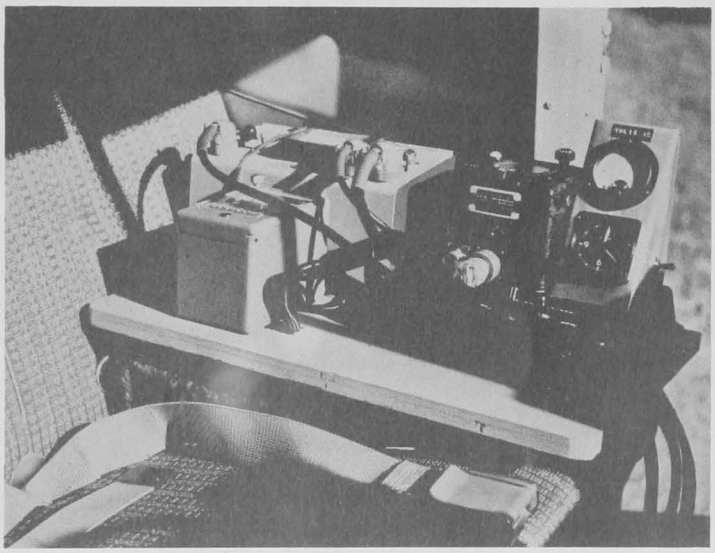
The purpose of the experiment was to determine the effectiveness of the sea bed drifter in Pacific coastal waters. Good success has been obtained on the Atlantic shelf with the devices, but bottom and hydrographic conditions along the west coast differ greatly from the Atlantic shelf. A total recovery of about 12 percent was achieved with this first experiment, giving not only clues to the bottom currents in Monterey Bay, but also to more effective use of the drifters.

An infrared temperature recorder has been installed in a light aircraft to determine if this method of obtaining surface sea water temperatures is accurate. This device will record temperatures continually at any speed of the aircraft providing temperature data for large ocean areas in a few hours. A ship would require several days or weeks to cover the same area, making it impossible to obtain essentially instantaneous records of the gradually changing temperatures.

Several transects have been made with this instrument along the California coast and in San Francisco and Tomales Bays. These have been compared with water temperatures surface recorded with mercury thermometers. It was found, as had already been determined, that fog or haze affects reading obtained, but that on clear days the instrument is quite accurate, particularly when flying at 500 feet elevation. From 1,000 feet the error amounted to as much as $\pm 0.5°$ F. It was concluded that this method of recording temperatures is probably at least as accurate as some conventional methods now being employed. In addition, temperatures over large areas can be recorded almost "instantaneously". Plans for bi-monthly coastwise temperature surveys are being formulated, and a proposal for continental shelf flights has been submitted to the U. S. Navy.

Photo at left:--Airborne infrared thermometer and accessory equipment; lower front, infrared optical detector head; upper left, infrared electronic circuity and temperature indicator; below, strip chart recorder; upper right, intervalometer for visual readout timing, clock and voltmeter.

Photo at right:--Airborne infrared thermometer and accessory equipment positioned in the light aircraft.



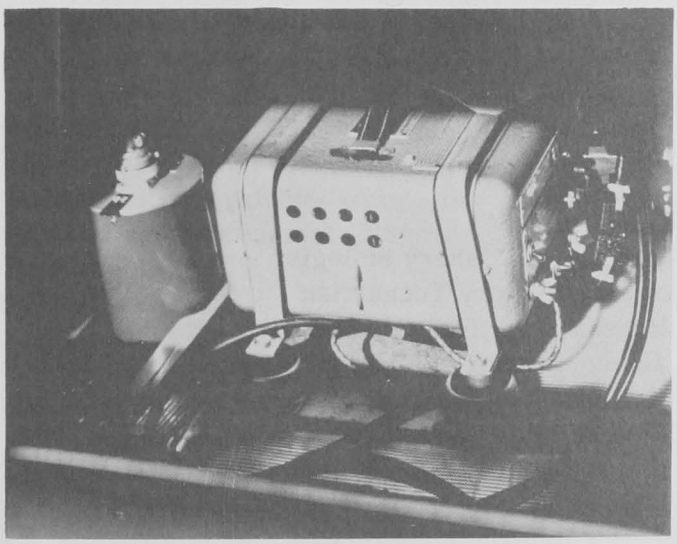


Photo at left:--Infrared detector head and power supply convertor shock-mounted on the aircraft luggage compartment floor. Detector looks vertically through a small hole in the bottom of the fuselage at the ocean's surface.

Arrangements have been made with four airborne fish spotters to record their observations of all species observed, their location, and an estimate of their abundance. These pilots normally report only schools of commercial fish to the catcher boats and direct fishing operations from the air. During their spotter trips they see, and can identify from the air, aggregations of anchovies, sardines, mackerel, barracuda, yellowtail, bonito, sea bass, sharks, rays, tuna, and several other species. Information on these species is being tape recorded and will be transcribed later at the laboratory. These data give observable relative abundance, distribution and movement of pelagic schooling species.

In late 1962 the U. S. Army Transportation Command officially released the T-boat T-437 to the Tiburon Marine Laboratory. This boat (65 feet overall, of all-steel construction, built in 1953, and powered with a 275 hp. Caterpillar V-eight diesel) will be modified for research in the coastal area.

Two-man dry submarine ordered for feeding and schooling behaviour studies. Photo by Arthur C. Madsen, Jr.

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Mr. Robert S. Kiwala, Fishery Technician
Mr. Edmund S. Hobson, Jr., Fishery Biologist
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Mrs. Walburga M. Reynolds, Clerk-Typist
Mr. Arthur C. Madsen, Maintenanceman

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Note: Underlined authors are not Branch of Fishery Research personnel.

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