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PROGRESS

IN SPORT FISHERY

RESEARCH

1964



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

Circular 210

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UNITED STATES DEPARTMENT OF THE INTERIOR, STEWART L. UDALL, SECRETARY
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Fish and Wildlife Service, Clarence F. Pautzke, Commissioner
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✓ PROGRESS IN SPORT FISHERY RESEARCH, 1964

✓ DIVISION OF FISHERY RESEARCH
Paul E. Thompson, Chief



DEPARTMENT OF FISHERY RESEARCH OF THE BUREAU OF SPORT FISHERIES AND WILDLIFE
LABORATORIES • STATIONS • RESEARCH STATIONS •
LOCATIONS OF ACTIVITIES
(Alphabetically by States. Where different, new offices are shown in parentheses. Figures refer to pages on which a 1964 reports began; the underlined figures indicate 1964 reports. In other figures indicate stations.)

Alabama: Marion, 55, 57.
Arkansas: Fayetteville, 75.
California: Coville Creek, 21.
Colorado: Denver, 59.
Georgia: Warm Springs, 24.
Idaho: Argonne, 20.
Maryland: Patuxent Center (Annapolis), 11.
Nevada: Reno, 41.
New Jersey: Sandy Hook (Highlands), 21, 69.
New York: Cortland, 13.
Oklahoma: Tishomingo, 49.
South Dakota: Yankton, 51.
Washington: Longview, 35, 69; Willard (Cock), 22, 69; Wattle, 2, 69.
West Virginia: Linton (Martinsville), 1.
Wisconsin: La Crosse, 24, 69.
Wyoming: Beulah, 105, 69; Jackson, 69.

U.S. Bureau of Sport Fisheries and Wildlife
Circular 210

Washington, D.C. • March 1965



DIVISION OF FISHERY RESEARCH OF THE BUREAU OF SPORT FISHERIES AND WILDLIFE

Laboratories ● Stations ● Proposed laboratories ○

LOCATIONS OF ACTIVITIES

(Alphabetically by States. Where different, post offices are shown in parentheses. Figures refer to pages on which activity reports begin; the underscored figures indicate laboratories and the other figures indicate stations.)

Alabama: Marion, 66, 69.

Arkansas: Fayetteville, 79, 87; Stuttgart, 48; Kelso, 48.

California: Convict Creek (Bishop), 41; Tiburon (Belvedere-Tiburon), 103.

Colorado: Denver, 69.

Georgia: Warm Springs, 74.

Idaho: Hagerman, 20.

Maryland: Patuxent Center (Laurel), 69.

Nevada: Reno, 41.

New Jersey: Sandy Hook (Highlands), 93, 69.

New York: Cortland, 13.

Oklahoma: Tishomingo, 69.

South Dakota: Yankton, 81.

Washington: Longview, 58, 69; Willard (Cook), 20, 69; Seattle, 9, 69.

West Virginia: Leetown (Kearneysville), 1.

Wisconsin: La Crosse, 74, 69.

Wyoming: Beulah, 106, 69; Jackson, 69.

Proposed: In Missouri, at Columbia, the new headquarters of the Fish-Pesticide Laboratory; in Rhode Island, at Saunderstown, the Narragansett Marine Laboratory.

CONTENTS

	Page
Introduction	iii
PATHOLOGY	
Eastern Fish Disease Laboratory	1
Western Fish Disease Laboratory	9
NUTRITION	
Eastern Fish Nutrition Laboratory	13
Western Fish Nutrition Laboratory	20
HUSBANDRY METHODS	
California-Nevada Sport Fishery Investigations	41
Fish Farming Experimental Station	48
Salmon-Cultural Laboratory	58
Southeastern Fish Cultural Laboratory	66
PESTICIDES	
Fish-Pesticide Research Laboratory	69
CONTROL	
Fish Control Laboratories	74
RESERVOIRS	
National Reservoir Research Program	79
North Central Reservoir Investigations	81
South Central Reservoir Investigations	87
MARINE	
Sandy Hook Marine Laboratory	93
Tiburon Marine Laboratory	103
GENETICS	
Fish Genetics Laboratory	106
TECHNICAL COMMUNICATION	107

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PROGRESS IN SPORT FISHERY RESEARCH, 1964

INTRODUCTION

PUBLICATION OF THIS REPORT is intended to "portray with accuracy and timeliness the frontiers of scientific research"^{1/} in Bureau laboratories during the past year.

It seems appropriate in introducing this progress report to describe the anatomy of sport fishery research to suggest how its organic systems (its people, laboratories, and programs) are interrelated and interdependent.

At mid-year, the staff was 205 persons of whom 115 were professionals and 90 were supporting. Academically speaking, 29 of the professionals had PhD's, 33 an M. S. or M. A., 20 had graduate work beyond the B. S., and 33 had a B. S. Professional specializations included 68 fishery biologists, 14 chemists, 6 biochemists, 3 physiologists, 1 entomologist, 4 histopathologists, 5 microbiologists or bacteriologists, 5 parasitologists, 3 pathologists, and one each in mathematical statistics, genetics, animal husbandry, oceanography, hydraulic engineering, and electrical engineering.

Seventeen laboratories and 13 field stations in 17 States had about 145,000 square feet of research and auxiliary space, exclusive of outdoor ponds, raceways, and minor supporting facilities.

Fish-culture related research received 36.5 percent of research funds (Pathology, 9.9 percent; Nutrition, 11.4 percent; and Husbandry Methods, including selective breeding, 15.2 percent). Fish-pesticide research has 23.4 percent of the total; fish control research, 11.6 percent; reservoir research, 13.1 percent; and marine game fish, 15.2 percent.

Professional employees were distributed among the research activities like this:

Pathology 13.9 percent	Fish control 11.3 percent
Nutrition 13.9	Reservoirs 13.9
Husbandry 22.6	Marine 11.3
Pesticides 9.6	Washington office	.. 3.5

Research cost per professional was a little less than \$19,000. In other research organizations this cost ranges from \$15,000 to \$30,000.

Supporting staff per professional is 0.7, or somewhat lower than the 1.5 we consider optimum. The 0.7 figure does not take into account, however, seasonally employed field and laboratory assistants and student trainees who may give some time as laboratory aids.

Technical communications per professional were 1.3. These included published papers, papers accepted and in press, and major technical addresses. The average cost per technical communication was \$14,500. Current comparisons are not available, but in 1961 the National Science Foundation reported industrial research publication costs ranged from \$38,000 to \$121,000, with an average of \$57,000.

^{1/} Shannon, James A. 1964. Recognizing technical communication needs. In *Technical Information and the Federal Laboratory*. Federal Council for Science and Technology.

Mechanisms of inter-laboratory and inter-disciplinary research are a functional part of the anatomy we are describing. These are too intricate to relate in detail. Some are formal, more are informal arrangements between laboratory directors or individual staff members, and some involve working relations with scientists in institutes and universities. A few illustrations will suffice to illustrate.

The Fish-Pesticide Research Laboratory at Denver has inter-laboratory arrangements with the Denver and Patuxent Wildlife Research Centers and with the Western Fish Disease, Salmon-Cultural, Western Fish Nutrition, Sandy Hook Marine, Southeastern Fish Cultural, and Fish Control Laboratories.

The Eastern Fish Nutrition Laboratory at Cortland, N. Y. works with Cornell University and the New York State Conservation Department.

Tiburon Marine Laboratory in California is rearing salmon fingerlings, the survivors of a virus disease epidemic, in its salt-water system for the Western Fish Disease Laboratory at Seattle.

Sandy Hook Marine Laboratory, Highlands, N. J., through its sponsoring of the Committee for Scientific Exploration of the Atlantic Shelf (SEAS), has continuous association with the coastal marine laboratories, coastal universities, the National Oceanographic Data Center, National Science Foundation, Geological Survey, Smithsonian Institution, Office of Naval Research, and Public Health Service.

The Western Fish Nutrition Laboratory, Cook, Wash., has working relations with the National Institutes of Health and at least six major universities with particular reference to dietary carcinogens and mold toxins.

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PATHOLOGY

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

HIGHLIGHTS

An international conference on viral diseases of poikilothermic vertebrates, organized jointly with the New York Academy of Sciences, was held in September in New York.

Bacterial gill disease was experimentally induced and transmitted to healthy stressed fingerling rainbow trout.

A description of bullfrog tongue fibroblasts, now 3-1/2-years old and in 73rd passage, is the first report of a continuously cultivable or permanent cell line from an amphibian.

Large-scale viral screening of presumptively infectious pancreatic necrosis (IPN) virus-free rainbow trout broodstock at two national fish hatcheries showed no evidence of this virus.

Lymphocystis virus has been unequivocally isolated and propagated in vitro. Cells in infected cultures had all the essential microscopic characteristics seen in lymphocystis cells from fish.

Five centrarchid cell lines were started; one could not be carried in serial sub-culture, but four are several months old and in seventh to twelfth passage.

Whirling disease of trout caused by Myxosoma cerebralis has been established for experimental purposes, although no experimental laboratory infections per se have been accomplished.

The experimental fish hosts of the black-spot strigeoid, Uvulifer ambloplitis were determined.

European trichodinids were described from North American goldfish.

The development of Myxosoma cartilaginis in the cartilage of the bluegills was described.

A method for testing the effect of chemicals on Ichthyophthirius was devised.

The following new species of fish parasites were described: Gyrodactylus from the bluegills, Dactylogyrus from the fallfish, Plistophora from shad and trout, Eimeria from the goldfish, Sanguinicola and Cryptobia from dace.

Work on residues of drugs in fish tissues was initiated.

BACTERIAL DISEASES

Aeromonas and vibrio

In our search for better and quicker methods of identifying and separating these closely related fish pathogens we investigated the use of various amino acid decarboxylases and also agar gel diffusion. Preliminary work with the decarboxylases was not encouraging and has been dropped. Preliminary work with agar gel diffusion using antiserum prepared in rainbow trout showed some common antigens among the aeromonads. This seemed a good tool for identifying the aeromonads and also for determining common antigens between aeromonas and vibrio. Extracellular and cell-free extract antigens were prepared from both an aerogenic and anaerogenic aeromonad and sent to Microbiological Associates, Inc., Bethesda, Maryland, where rabbit antiserum was prepared from each antigen. At present aeromonad and vibrio cultures are being tested against each serum using both agglutination and agar gel diffusion precipitin tests.

Chesapeake Bay fish kill

Work on the Pasteurella from white perch has been completed and a note published in the Journal of Bacteriology. Serological tests per-

formed by Drs. Hansen and Marshall at the University of Maryland also place this bacterium in the Pasteurella genus. No one has been able to define the bacterium to the species level. Koch's postulates have been completely fulfilled with the finding by University of Maryland workers that the bacterium produced an experimental infection in white perch upon injection. No white perch kill was reported during the 1964 summer.

Encapsulated bacterium from goldfish

A paper dealing with the identification and pathogenicity of the bacterium has been accepted by Applied Microbiology. Perhaps the most interesting feature of the organism was its ability to produce an experimental infection in goldfish simply by exposing fish with a few scales removed to a suspension of organisms.

Substitute medium for Hemophilus piscium

Mueller-Hinton Medium (Difco Laboratories, Detroit, Michigan) containing 2 µg/ml of cocarboxylase was found to support the growth of stock cultures of H. piscium most satisfactorily. A report is in press.

Fish pathogenic myxobacteria

Bacterial gill disease was repeatedly induced in 3-5 inch stressed rainbow trout fingerlings, while no gill disease could be induced in control trout. Stressed fingerlings have also been infected by addition of infected fingerlings. We have no success in inducing gill disease in larger rainbow fingerlings. Stress factors were crowding (ratio of 1/15 fish/water), an oxygen level of 3.5-4.5 ppm and an ammonia level of .1-1.5 ppm. Control trout were crowded but oxygen levels were 7.0-7.5 ppm with only a trace of ammonia.

In addition to myxobacteria, motile aeromonads and pseudomonads have been repeatedly isolated from trout with gill disease. An attempt will be made to understand the role of these bacteria as well as the gill myxobacteria.

To test the ability of gill myxobacteria to infect trout by injection, and also to compare with other myxobacteria, two lots of rainbow trout were injected intramuscularly and intra-

peritoneally with suspensions of three myxobacterial cultures. Rainbow trout averaged 18.8 grams in one lot and 12.3 grams in the other. Numbers of bacteria injected and mortality of rainbow trout after 21 days are given below:

	<u>Peduncle Disease</u>	<u>Gill Dis- ease</u>	<u>Cold- water Disease</u>	<u>Control</u>
Approximate number of bacteria injected/trout	6 x 10 ⁸	6 x 10 ⁸	10 ⁶	--
Mortality in small rainbows	24/40	19/40	3/40	0/40
Mortality in larger rainbows	8/40	4/40	0/40	0/40

Pathology resulting from infection was the same for all cultures, namely hemorrhagic areas in the gill covers and base of fins externally, and extensive hemorrhagic areas in body wall, viscera and at times gonads. The intestine was flaccid and filled with thick yellow fluid. Since mortality usually came rapidly, attempts were made to demonstrate exo- or endotoxin. All attempts failed. Myxobacteria were cultured from kidneys of infected fish. The results indicate that gill disease myxobacteria like peduncle disease myxobacteria can produce disease when injected in very large numbers into trout, especially small trout. The few fish dying from injection of coldwater disease myxobacterium may have been a result of too few organisms injected as compared with the other two cultures.

VIRAL DISEASES OF FRESHWATER FISHES

Possible virus etiology of rainbow trout hepatoma

Nutritional research has so convincingly demonstrated that rainbow trout hepatoma is largely if not exclusively a result of dietary carcinogens that virological research will not be pursued. The work is terminated.

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Serological identification of infectious pancreatic necrosis (IPN) virus

The practical identification of IPN virus by means of agar gel diffusion has not yet been realized. Hyperimmune rabbit anti-IPN sera prepared with cell culture grown virus have antibodies not only against the virus but also against cellular and serum proteins. Precipitin reactions involving cellular and serum proteins were readily demonstrated, but the viral antigen reaction could not be seen. It appears likely that the absence of a visible reaction stems from too little viral antigen. The IPN virus measures only 18.5 μ in diameter and is among the smallest known viruses.

At this time, the cell culture neutralization test remains the best serological test for identification of IPN virus.

Role of the male in transmission of IPN virus

Individual sera from 14 male brook trout from a population containing IPN "carriers" were tested for neutralizing antibody. As tested against approximately 500 ID₅₀, the sera had titers of 1:4 to 1:512. The same sera will be tested for bacterial agglutinating antibody.

Control of IPN

IPN-free individuals from a population of "carrier" brook trout were selected by virological examination and then spawned. Their offspring showed only slight loss from IPN and are now over a year old. This work demonstrated a practical approach to control of IPN and similar diseases transmitted with fish eggs.

In order to avoid such egg-borne problem diseases, similar specific pathogen-free fish should be propagated in water from wells or enclosed springs which can harbor no disease carriers. There are such hatcheries which are not now used for broodstock purposes.

The principle of propagating specific pathogen-free stock has been applied in the southeast. The National Fish Hatchery at New Castle and the old Wytheville National Fish Hatchery, Virginia have rainbow brood-

stock with no history of IPN. Fishery research and fish hatchery personnel cooperated in screening a 10-percent sample of all broodstock for evidence of IPN virus. Pooled peritoneal washes were tested in replicate rainbow trout gonad (RTG-2) cell cultures, and no evidence of IPN virus was found. In view of their particular history and results of the screening, the fish can be considered as certified IPN-free stock, and all efforts should be bent to maintain them as such. Until such time as other certified sources of eggs are established, this means that neither eggs nor fish should be admitted from other stations.

Commercial trout growers have requested advice on the general problem of controlling egg-borne diseases, and initial reactions towards possible certification of stock are positive.

Lyophilization of IPN virus

Unexpectedly, lyophilized IPN virus showed progressive decline in infectivity during a period of 3 months. Accordingly, a second test was started to measure infectivity over a longer period of time. The first portion of the second test confirmed the earlier work; highest levels of infectivity were found in preparations incorporating lactalbumin hydrolysate, beef liver, skim milk or lactose.

One-step growth curve of lymphocystis virus in the bluegills

Multiplication of lymphocystis virus in bluegills was studied at 25°C. during a period of 30 days. From its initial level, infectivity declined in eclipse phase and virus could not be demonstrated at day 4. Virus was found again at day 6 and since its level exceeded the initial level it was assumed to be new virus. Exponential growth of virus occurred through day 12, and after this level was reached, little more virus was produced. A manuscript has been prepared and the work has been reported.

RESEARCH ON COLD-BLOODED ANIMAL CELL AND TISSUE CULTURE

Additional methods for cell culture

The line of fibroblast cells which was started from adult bullfrog tongue (FT) tissue more than 3-1/2 years ago is in seventy-third subculture. Chromosome analysis was completed, and the modal number was found to be $3N+3$, a hypertriploid condition. Characterization was completed and a description of the cell line has been published. Cultures have been turned over to a collaborating laboratory for preparation and deposit in the Cell Repository of the American Type Culture Collection. The work unit is completed.

The work of maintaining cell lines has been reduced by use of low temperature frozen storage. Antibiotic-free stocks of RTG-2 and FT cells have been prepared in part with dimethylsulfoxide and in part with glycerol and then frozen. Storage is at -80°C .

Stock cultures of fish and frog cells have been sent to Yugoslavia, France, and Canada. There have been 31 shipments of cells to laboratories within the United States.

Development of a permanent line of cells from a centrarchid fish

Three lines of cells from largemouth bass were established for the purpose of developing a permanent line of cells from a centrarchid fish. Such a cell line is a logical system to use in searching for virus as a cause of problem diseases in this important family of hatchery propagated fishes. The cells are 5 and 6 months old, and the most advanced line is in eleventh subculture.

Similar cultures were also started from bluegills. Although somewhat younger, the bluegill cells are proving more active than the bass cells.

The centrarchid cells are susceptible to both IPN and lymphocystis virus (fig. 1).



Figure 1:--A 13-day-old lymphocystis cell produced in vitro by second transfer of cell-grown lymphocystis virus. Such cells have essential attributes of lymphocystis cells found in natural infections: (1) large size - this cell is $104\ \mu$ in length, (2) pronounced basophilia, (3) enlarged nuclei and nucleoli (trio of small solid arrows), (4) large basophilic inclusions in the cytoplasm (large open arrows). Thick hyaline capsules develop after additional maturation but their thickness obscures internal detail. The two large solid arrows point to normal nuclei in adjacent uninfected cells.

PARASITOLOGY

Whirling disease of trout

Whirling disease caused by Myxosoma cerebralis (Protozoa: Myxosporidia) is still a problem in the northeastern States. Control, but not eradication, has been achieved by four hatcheries. Because eradication has been impossible the disease has continued to spread to other hatcheries, mainly through fish transfers.

In further attempts to experimentally infect fish, "aged" spores were introduced into the stomachs of rainbow trout fry. Russian researchers had reported success in this method, but of 100 fish we treated, none became infected.

The disease has been maintained for experimental purposes at Leetown in a unique way: two small lots of infected yearling and 2-year-old rainbow trout were held in facilities which drained into the Leetown Run. In June one small lot of fry was infected by holding them in a live-box in the run. In August another lot was likewise infected. It is probable that the infective spores came from the fish being so held in the laboratory. If so, this supports the Russian hypothesis that spores are passed by living infected fish. Because it has been assumed that small invertebrates serve as "carrier" hosts, we collected many tubificid worms from the contaminated area and fed them to small trout; none became infected. Experiments have been started to determine if the contamination is still present and the youngest age at which trout can be infected. Work is planned to determine the "carrier" host.

Peritrichous protozoa

A laboratory epizootic of trichodiniasis killed a trough of bluegills, pumpkinseeds and smallmouth bass. Later it caused mortalities in a trough of bluegills that had been maintained for 5 years. We exposed suckers, dace, catfish, goldfish, shiners, green sunfish, rainbow trout and brook trout to the parasite. At 7 days all were heavily infected; by 18 days all five brook trout, one of three suckers, and the one catfish were still heavily infected and died. At 30 days only the two suckers and one dace were still infected. Apparently the remainder had developed some sort of resistance.

Epizootics of the same Trichodina occurred three times later in the laboratory. Apparently the environment, which includes 12°C. water, favors this particular species. The late Dr. H. S. Davis reported it from Leetown in 1947 and it has apparently been here ever since.

A paper by Dr. Jiri Lom, Czechoslovakia, and Dr. Glenn L. Hoffman, dealing with fish pathogenic trichodinids was published in the Journal of Parasitology. Two European species were found on North American goldfish; T. fultoni was redescribed and T. domerguei f. magna, previously described from European

tench, was considered a synonym. Dr. Lom is collaborating with us on further trichodinid work.

Myxosoma sp. in the cartilage of bluegills

This new species, M. cartilaginis, was described and the development elucidated. The sporoplasm invades head cartilage primarily, and becomes a multinucleate trophozoite which forms pansporoblasts, each of which produces two to four spores. The first spores appear in 7 weeks. The histopathology consists at first of little cellular reaction, but after 4 to 5 months epithelioid granulomas appear around some of the spore masses. Cartilage liquefaction is present around the parasites for at least 5 weeks. Eosinophilic globules are present in cartilage cells adjacent to the lesions. Diffuse infiltration of the spores from the lesions was described.

Of 24 chemicals tested for polar filament extrusion, potassium hydroxide gave the best results.

An illustrated synopsis of the Myxosoma of North American fishes was prepared which included additional information and illustrations of M. hoffmani Meglitsch, 1963. Also included was a table showing the host, site of infection, geographic location, spore and polar capsule sizes. A report was accepted for publication.

Microsporidea of fish

Two new species of Microsporidea, Plistophora salmonae from steelhead and rainbow trout and P. cepedianae from gizzard shad were described. Schizonts to spores of P. cepedianae were found at one time within the same cyst, while only sporonts and spores of P. salmonae were found within the cyst. An illustrated synopsis of the known Microsporidea of freshwater and euryhaline fishes was prepared. A manuscript has been accepted for publication by the Journal of Protozoology.

Control of Ichthyophthirius

A method of testing the effectiveness of chemicals on the free-living stages of Ichthyoph-

thirus has been devised. Preliminary testing of the following has been accomplished: atabrine, acriflavine, ammopyroquin dihydrochloride, CoRal, Flagyl, formalin, malachite green, methylene blue, nickel sulfate, Néguvon, pyridyl mercuric acetate (PMA), quinine hydrochloride, Roccal, TV-1096 (Parke, Davis), Tiguvon and sodium chloride.

Of these, formalin, Néguvon, TV-1096, methylene blue, ammopyroquin dihydrochloride, quinine hydrochloride and PMA show the most promise for prolonged treatment of 24 hours or more at dilute concentrations (0.1 to 2 ppm usually). Fish toxicity studies done by the Fish Control Laboratory indicate these could be used for prolonged treatment to kill the free-living stages of Ichthyophthirius.

Ichthyophthirius is still being maintained in the laboratory. Work on the immunization of trout against Ichthyophthirius by controlled infection with the organism was continued on a larger scale. Three large tanks were stocked with 500 rainbow fingerlings each. Fish in tank I were exposed to about 2,000 tomites per fish and at the first sign of visible ichthyophthiriasis fish were treated daily with 1:4,000 formalin until the infection disappeared. Tanks II and III served as controls. A month later tank I was given a challenge dose of about 2,500 tomites per fish with tank II serving as a control at similar dosage. Tank II developed a moderate infection while tank I showed no visible Ichthyophthirius. Two months later tanks I and II were challenged and tank III was given a similar dose. Before results could be gained from this final step a heavy Gyrodactylus infection occurred as well as low water and low oxygen resulting in the loss of about 1,000 of the initial experimental 1,500 fish. Treatment against the Gyrodactylus infection was not possible since we do not know of a selective treatment which will kill Gyrodactylus and not Ichthyophthirius. When the Gyrodactylus reached its peak, no visible signs of Ichthyophthirius could be found. We hope to continue this work when suitable space and a parasite-free water supply is available.

As an indication of the possible importance of immunity, the Bowden Springs National

Fish Hatchery has had epizootics of Ichthyophthirius disease annually for several years, but the yearlings which survived the 1963 epizootic did not become diseased during the 1964 epizootic, although they received water from the contaminated raceways. This indicates effective immunization.

Diplostomulum from the eyes of hatchery catfish

In a study of the life cycle of this parasite, parasite-free gulls were experimentally infected and adult trematodes were recovered. The trematode was identical with Diplostomum spathaceum (D. flexicaudum). Trematode eggs were recovered, incubated, and exposed to laboratory reared snails (Lymnaea jugularis) -- none became infected although lymnaeid snails have been reported as hosts of this species.

Parasitic algae in bluegills

Unicellular algae were found in bluegills from a nearby pond on two occasions. On the first occasion they were found in cysts in the eye orbit; and the alga was identified as Chlorella sp.; a manuscript describing this is in press with The Progressive Fish-Culturist. On a later occasion many small cysts, containing algae, were found by Mr. Tim Bowen, trainee, in the gills of one fish. Dr. G. W. Prescott, Michigan State University, examined a sample of the material and stated that it would have to be called Chlorococcum infusioinum with reservations. Ten fish were injected with this "parasite" and living algae were recovered from two. There was no evidence of reproduction or spreading of the alga, however.

Sanguinicola, blood fluke of fish

A new species of blood fluke was found in the gill arch blood vessels of cyprinids by Dr. Joe Hunn, research fellow. It will be studied and described as a new species.

Cryptobia in West Virginia fish

An apparently new species of blood parasite of fish (Cryptobia) was found by Mr. Charles Carlson, trainee. The host range is being

studied by experimental infection and it will be described as a new species. Attempts are being made to grow the organism in artificial media, and to preserve it by freezing.

HISTOPATHOLOGY

Visceral granuloma

This experiment, designed to induce visceral granuloma in brook trout by means of diet, is progressing as planned. Halver's complete test diet (CTD) used as a base diet has produced good growth of fish and the physiological parameters measured indicate it is adequate for good health.

The experiment has been in progress for 8 months and for the last 5 months samples have been made monthly. The samples consist of two fish each and several tissues were sectioned from each fish to determine the presence or absence of granuloma. The hematocrit and plasma protein readings were obtained from the fish sampled in November and December. The readings vary considerably between diets, but only those fish receiving gossypol are considered below normal. Results to date, summarized in table 1, strongly implicate diet as a factor contributing to the development of this disease.

Table 1:--Dietary induction of visceral granuloma in brook trout

<u>Diets</u>	<u>No. fish examined</u>	<u>No. fish with granuloma</u>	<u>Hematocrit</u>		<u>Plasma Protein Grams percent</u>	
			<u>Nov.</u>	<u>Dec.</u>	<u>Nov.</u>	<u>Dec.</u>
Control Diet Halver's Complete Test Diet (CTD)	10	0	40	42	4.3	4.7
Cottonseed Meal Diet CTD + 22 percent Com- mercial cottonseed meal	10	2	39	41	4.0	3.6
Fat Diet Corn oil of CTD replaced with beef tallow	10	1	43	43	3.6	3.2
Gossypol Diet 100 mg gossypol/100 gr dry wt. of CTD	10	0	33	33	4.5	3.2
Cortland No. 6*	10	10	44	37	4.3	4.2

*Same as the original Cortland No. 6 formula except poultry type fish meal is used in place of vacuum dried white fish meal.

DIAGNOSTIC PROCEDURES

Paper electrophoresis

The story of some patho-physiologic effects of corynebacterial kidney disease on brook trout has been completed and published.

Mimeographed copies of a bibliography entitled "Electrophoretic Investigations of Fish Blood and other Body Fluids", have been sent to workers active in the field for additions and corrections. This work is being carried out by Dr. J. B. Hunn who is a fellow of the National Institutes of Health.

Determination of drug residues in trout tissues

Final preparations are being made for the presentation of a petition to Food and Drug Administration requesting clearance of sulfamerazine as a fish food additive. The American Cyanamid Company has granted permission to refer to their master file for any necessary information. Agway Inc., has agreed to furnish necessary information on incorporation of the drug into pelleted diets. Final muscle residues determined by Diablo Laboratories on muscle samples taken 14, 20, 26 and 30 days post treatment showed a mean background of .02 ppm sulfamerazine for rainbow trout and .03 ppm for brook trout. The analytical procedure used by Diablo Laboratories was an ion exchange method recommended by the Food and Drug Administration. Preliminary statistical analysis indicates a 30-day withdrawal period may be required if it is necessary that no residue be present. In the near future we expect to receive additional data from the cooperating State laboratories in Wisconsin, Minnesota and Michigan.

DIAGNOSTIC SERVICES

Histopathological examinations were made on 108 cases referred to the laboratory by hatchery biologists and others.

In 1963 and again in 1964 Atlantic salmon at the Craig Brook station suffered high mortality. In both instances a yeast-like organism was isolated in pure culture and also seen in

large numbers in histological sections of stomach and hindgut. The organism was identified by mycologists as belonging to the genus Candida, a yeast-like fungus. The fungus was cultured in great numbers in beef liver fed the fish and also in the feed preparation room. There is no direct evidence, however, that this organism is pathogenic for the Atlantic salmon fingerlings.

PREPARATION OF TEXTS AND SPECIAL LECTURES

Oral presentations

Dr. Snieszko was chairman and Dr. Wolf cochairman of the symposium on Virus Diseases of Poikilothermic Vertebrates held at the Waldorf Astoria Hotel, New York City, September 23-26, 1964.

Dr. Wolf was chairman for the session on "Fish culture and diseases of fish", 94th annual meeting of the American Fisheries Society, Atlantic City, New Jersey, September 14-16, 1964.

Parasites of North American freshwater fishes

Rough drafts of the algae, fungi, protozoa, monogenetic trematodes, adult digenetic trematodes, metacercarial trematodes, cestodes, nematodes and acanthocephala have been completed. Remaining are the copepods, host list and illustrations. If enough typing can be accomplished it is hoped that the final draft of this book can be completed in 1965. The University of California Press has tentatively accepted it for publication.

STAFF

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WESTERN FISH DISEASE LABORATORY

Seattle, Washington
Robert R. Rucker, Director

HIGHLIGHTS

Sacramento River chinook disease (SRCD), Oregon sockeye disease (OSD), and infectious pancreatic necrosis (IPN) pathogenesis, immunopathogenesis, and epizootiological studies were completed and the data presented at the New York conference on the Viral Diseases of Poikilothermic Vertebrates.

SRCD pathology was observed in chinook salmon sac fry.

Pathology resembling the old "Leavenworth Sockeye Virus Disease" was recovered from adult sockeye salmon.

A hepatoma survey was completed.

Cellular changes resembling neoplasia were noted in an unusual number of moribund fingerling coho salmon.

In the West, more and more attention is being focused on IPN in commercially-raised rainbow trout.

Rainbow trout in a hatchery situation were orally immunized against redmouth disease.

The testing of various antibacterial drugs for their effectiveness against some infectious diseases of salmonids is continuing.

BACTERIOLOGY

An Aeromonas sp. isolated from yellow perch during a fish kill in a Montana lake several years ago was tested for host specificity. Sockeye salmon, rainbow trout, squawfish, and yellow perch were exposed to the organism by turning off the water supply, adding viable cultures of the bacteria to the water in the aquaria, then turning on the water one hour later. The majority of the perch died and homotypic bacteria were isolated from the kidney of each dead fish. None of the other species died of the disease and,

at conclusion of the study, no organisms were cultured from the kidney of any remaining fish. The control fish showed no evidence of disease throughout the course of the study. Pathogenetic description of "bacterial perch disease" is being undertaken. To provide material, one group of perch was infected by exposing them to a suspension of the organism in the water; the control group was exposed to a portion of the broth medium in the water. Histological and hematological samples are being taken at weekly intervals for 8 weeks.

Twenty-five experimental sulfa compounds and 23 substituted salicylanilides were tested for growth inhibition of 32 strains of myxobacteria, among which were Chondrococcus columnaris, Cytophaga psychrophila, and isolates from cases of bacterial gill disease. At the concentration of 20 micrograms of drug per milliliter of medium, only 3 of the substituted salicylanilides inhibited growth of the organisms. These were retested at levels of 1.0, 0.1, and 0.01 microgram of the drug per milliliter of medium. Terramycin at the same levels proved more effective.

The 23 substituted salicylanilides were tested against 32 strains of bacterial fish pathogens, which included A. salmonicida, A. liquefaciens, Vibrio anguillarum, and the Idaho redmouth disease agent. At the level of 20 micrograms per milliliter of medium, none of the drugs was inhibitory.

Twelve relatively new or experimental drugs and antibiotics were tested for their ability to inhibit growth of Cytophaga psychrophila, the etiologic agent of cold-water disease. Mandelamine, one of three which showed promise, was fed at three dosage levels to experimentally infected coho salmon. The intermediate level, which showed favorable results, may be recommended for experimental testing on a small group of naturally infected fish.

A cooperative study with the Western Fish Nutrition Laboratory was conducted to

investigate the relation of dietary iodide and incidence of kidney disease in young chinook salmon. Groups of fingerling chinook salmon were fed varying levels of iodide in the complete test diet. The experiment was terminated before its planned conclusion due to a spontaneous epizootic of kidney disease in the hatchery. The expected relation between incidence of kidney disease and level of dietary iodide was not manifest at time of the epizootic.

Nineteen strains of *Aeromonas* spp., among which were cultures of *A. hydrophila*, *A. liquefaciens*, and *A. punctata*, were compared biochemically with 19 tests routinely used in bacteriological identification. Only 3 of the biochemical properties were common to all 19 strains tested.

A study was initiated to determine if a toxin is produced by *Chondrococcus columnaris* and, if so, its role in the course of infection of fish. Work at present is directed towards recovery of bacterial cell wall material.

A study was initiated to describe the pathogenesis of furunculosis in rainbow trout. One group of rainbow trout was injected subcutaneously with a saline suspension of *A. salmonicida*; trout injected subcutaneously with saline served as controls. Tissue, blood, and serum samples were taken at 8-hour intervals for the next 5 days. Examinations are continuing.

PARASITOLOGY

As of September 1 the parasitology section was dropped from the laboratory. Processing of samples of fish from managed lake environments is being continued under contract with the University of Washington's College of Fisheries. During the first 3 months of the study, 169 salmonids from managed lakes have been examined for parasites. A summary of findings is presented in tabulated form at the bottom of this page.

VIROLOGY

Infectious pancreatic necrosis (IPN) was confirmed in fish from two hatcheries never before known to harbor the disease. IPN incidence has been verified in hatchery fish from most of the 11 western States and is a matter of great concern to fish conservationists. It has not been possible to determine whether this disease was recently introduced to these western States and is spreading or whether it may have been present and unrecognized for some time. The problem, however, has excited broad consideration of the need for procedures to insure better recognition, and reporting and recording of the incidence of IPN and other serious or potentially serious fish diseases, and for assurance that such diseases will not be spread to other areas.

During the last quarter, a filtrable agent was isolated from liver samples from adult sock-eye salmon returning to spawn in the Wenatchee

Percent of salmonids infected with each of three cestodes.

Species	Number	Parasite*		
		(Percent of fish infected)		
		D.	P.s.	P.a.
Rainbow trout	99	43.4	68.6	12.1
Outthroat trout	17	47.0	11.7	11.7
Coho salmon	45	55.5	0.00	0.00
Eastern brook trout	8	100.00	10.00	50.0

- * D. - *Diphylllobothrium* sp.
 P.s. - *Proteocephalus salmonidicola*
 P.a. - *Proteocephalus ambloplitis*

River, Washington. This agent, referred to in a recent quarterly progress report as Columbia River sockeye disease virus, is now thought to be the old "Leavenworth sockeye virus". Present studies are concerned with determining its relation to OSD and other viruses. Tissue changes very similar to those associated with SRCD and OSD were seen in sockeye salmon fingerlings injected with a cell-free filtrate of livers from these Wenatchee River sockeye salmon. In these fish there was also liver necrosis, a lesion not associated with either SRCD or OSD.

Liver samples from 50 Chilco Lake, British Columbia, adult sockeye salmon were negative for a filtrable agent.

Chinook salmon from the Coleman National Fish Hatchery that survived an epizootic of SRCD were transported to the Tiburon Marine Laboratory to be maintained in a marine environment to maturity. We plan to study the progeny of these fish for evidence of SRCD. In addition, a closed salt water system was put into operation in our laboratory to conduct intermittent studies of fish being held at Tiburon and experimental fish obtained from laboratory studies. In conjunction with the current SRCD study and examination of samples of fish submitted for diagnosis, yolk sac fry having typical SRCD histopathology were seen. This is the first recording of naturally occurring SRCD in fish so young.

All the tissue cultures, including the 25th passage of the WF-D4 rainbow trout gonad cell cultures, were contaminated by Gram-negative organisms and had to be discarded. To continue the virus studies, RTG-2 and frog tongue (FT) cell cultures were obtained from Dr. Ken Wolf at the Eastern Fish Disease Laboratory. Also, to prevent a recurrence of the problem, tissue culture facilities and techniques were modified. New rainbow trout and salmon cell-line cultures have been started.

IMMUNOLOGY

Attempts at oral immunization of rainbow trout in a hatchery situation against the Idaho

redmouth disease agent were made at the Hagerman, Idaho NFH. Although the same degree of immunity seen in laboratory trials was not apparent, there was evidence of a significant degree of protection. Experiments designed to gain insight into antibody forming mechanisms were conducted. Results from these studies and the oral immunization trials are being evaluated for practical application in prevention of infectious diseases of hatchery-reared salmonids.

A kidney disease bacterin was prepared and mixed into commercial pellets at two levels. Each level was fed to a group of fingerling chinook salmon. Control fish received untreated feed. To date attempts have not been made to determine if there is any immunity against kidney disease.

GENERAL

The rainbow trout hepatoma survey, conducted by the Division of Fish Hatcheries for 28 months, was concluded during the first quarter of this year. Livers from 10-20 rainbow trout on each of four commercially prepared diets (all coded) were received from the Ennis, Montana; McNary, Arizona; and Quilcene, Washington, National Fish Hatcheries. The final report of histopathological findings has been submitted to the regional office.

"Whirling" by fingerling coho salmon was observed at one of the Columbia River national fish hatcheries. These fish, however, had other clinical manifestations of disease. Scoliosis, lordosis, and distended abdomens were the more common findings. Mortality attributable to these was not high. Abdominal distention was found due to an over-inflated air bladder. There were no organisms noted in any of the tissue sections. Extensive cellular changes suggestive of neoplasia were found in the area of the medulla oblongata. These changes were found in most fish having any or all of the clinical manifestations of the syndrome. Detailed morphological, pathological, and virological studies are being done on recently submitted samples.

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HIGHLIGHTS

At the end of 15 months, three new pelleted fish foods prepared by the research results and fish department.

Fish growth was maintained on three new protein diets provided the caloric content of the diet was retained.

Level of cholesterol in trout blood was correlated with age and, in mature fish, sex.

Both diet and water temperature affected blood cholesterol of immature brown trout.

Source of dietary protein altered the protein level of fish blood serum.

Vitamin E is an essential vitamin for brown trout.

A stomach tumor was constructed and tested.

Experimental evidence supports in part the postulate that trout grow at a constant rate in length when held at a constant water temperature.

Immediately after hatching, brown trout absorb less than 20 percent of the amount of dissolved calcium that was absorbed by eggs immediately before hatching.

Brown trout fingerlings maintained satisfactory calcium regulation under stresses of water temperature and salinity when held in hard water.

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

Effect of three new pelleted fish foods, low protein, low caloric and addition of anti-oxidants to a complete dry food for trout

Three new pelleted fish foods (Nos. 4, 5, and 6) have been fed to duplicate mixtures of trout held under production conditions of the hatchery for 14 months. Pellet No. 4 is a low protein (32.5 percent) low caloric (895 per pound) food, No. 5 is a low protein (12 percent) high caloric (1,026 per pound) food, and No. 6 is a high protein (42.6 percent) and high caloric (1,191 per pound) pellet.

Fish fed the No. 6 pellet grew at the fastest rate (average weight 0.707 pounds) and disease had less effect. Pellets 4 and 5 yielded almost identically (average weights 0.545 and 0.541 pounds). The No. 5 pellet was the most efficient producer of fish flesh requiring only 1.36 pounds of pellets per pound of fish produced, the No. 4 pellet was second requiring 1.71 and the No. 6 pellet last requiring 1.87 pounds.

In terms of calories required per pound of fish produced the No. 4 pellet was most efficient (1,436 calories per pound of fish produced), the No. 5 pellet second (1,761 calories) and the No. 6 pellet last (1,887 calories). The No. 5 pellet was more efficient in utilization of dietary protein, producing a pound of fish for each 240

NUTRITION

EASTERN FISH NUTRITION LABORATORY

Cortland, New York

Arthur M. Phillips, Jr., Director

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". The Cortland reports are complete descriptions of the research results and may be obtained from the laboratory or the New York Conservation Department.

HIGHLIGHTS

At the end of 18 months, newly formulated pelleted fish foods continue satisfactorily as complete trout diets.

Fish growth was maintained on three low protein diets provided the caloric content of the diet was retained.

Level of cholesterol in trout blood was correlated with age and, in mature fish, sex.

Both diet and water temperature altered blood cholesterol of immature brown trout.

Source of dietary protein altered the protein level of fish blood serum.

Vitamin E is an essential vitamin for brown trout.

A stamina tunnel was constructed and tested.

Experimental evidence supports in part the postulate that trout grow at a constant rate in length when held at a constant water temperature.

Immediately after hatching, brown trout fry absorbed less than 20 percent of the amount of dissolved calcium that was absorbed by eggs immediately before hatching.

Brown trout fingerlings maintained satisfactory calcium regulation under stresses of water temperature and salinities when held in a hard water.

Significant distribution and storage of dietary calcium in both skeleton and skin of brown trout were measured soon after feeding.

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

Effect of changes in the vitamin package, energy source, and addition of anti-oxidants to a complete dry food for trout

Three new pelleted fish foods (Nos. 4, 5, and 6) have been fed to duplicate raceways of trout held under production conditions of the hatchery for 18 months. Pellet No. 4 is a low protein (32 percent) low calorie (875 per pound) food; No. 5 a low protein (32 percent) high calorie (1,024 per pound) food; and No. 6 a high protein (43.6 percent) and high calorie (1,184 per pound) pellet.

Fish fed the No. 6 pellet grew at the fastest rate (average weight 0.707 pounds) and those fed Nos. 4 and 5 pellets gained almost identically (average weights 0.588 and 0.581 pounds). The No. 6 pellet was the most efficient producer of fish flesh requiring only 1.56 pounds of pellets per pound of fish produced, the No. 5 pellet was second requiring 1.71 and the No. 4 pellet last requiring 1.87 pounds.

In terms of calories required per pound of fish produced the No. 4 pellet was most efficient (1,636 calories per pound of fish produced), the No. 5 pellet second (1,741 calories) and the No. 6 pellet last (1,847 calories). The No. 5 pellet was most efficient in utilization of dietary protein, producing a pound of fish for each 245

grams of protein fed; No. 4 was intermediate at a rate of 272 grams of protein; and No. 6 was least efficient requiring 313 grams of protein per pound of fish produced.

Fish mortalities have been normal for this hatchery and there have been no obvious nutritional disorders.

Growth of brook trout fed protein

Three dry meal mixtures containing different major sources of protein (fish meal, meat meal, or soybean meal) were added to fresh meat so that resultant diets had approximately the same percentage of protein (27 percent) and same level of calories (675 calories per pound).

At the end of the 20-week experimental period trout fed the diet containing fish meal or meat meal produced fish of approximately equal average weights (22.8 and 21.9 grams). Fish fed the diet containing soybean meal were much smaller (16.8 grams).

Calorie content and protein level of the three diets was reduced by dilution with inert cellu-flour so that all three diets contained approximately 445 calories per pound and 18 percent protein. As expected, trout fed these diets grew at reduced rates (average weights of 13.4, 13.8, and 12.9 grams for those receiving fish meal, meat meal, and soybean meal, respectively).

Increasing the caloric content of these diets with corn oil to levels similar to the controls but maintaining the reduced protein level restored the growth rate of the fish to those of the controls. The diets maintained the same relative positions of the controls in that the diets containing the fish meal or meat meal produced trout significantly heavier (25.5 and 23.4 grams) than that containing the soybean meal (17.8 grams). Proximate analyses of the trout showed that not all increased gain was synthesized protein since much was in the form of body fat.

In these tests there was little to choose between diets containing meat or fish meal but the diet containing soybean meal was inferior.

CHEMICAL COMPOSITION OF HATCHERY AND WILD TROUT

Chemical composition of trout fed natural foods and hatchery diets over a growing season

Brook trout fed either a meat-dry meal mixture or freshwater scuds (genus *Gammarus*) were analyzed at the start and end of a 3-month experimental period. There were no differences in body chemistry (proximate analyses) at the end of the experiment. Brook trout fed ground earthworms over a similar period did show a difference in proximate analyses of the body in that those fed earthworms had a lower fat content.

In both experimental groups it required nearly twice as many calories and one-third more protein to produce a pound of fish with the meat-dry meal mixture than it did with either of the natural foods. These results are similar to those previously found.

CHEMICAL COMPOSITION OF TROUT BLOOD

Determination of the effect of age and sex on serum cholesterol of brown trout

Three ages of brown trout (1-, 2-, and 3-year-old fish) were held separately in a similar environment for approximately 2.5 months and were fed a diet of meat and dry meals. At the end of this period their blood serum was analyzed for cholesterol.

There was significant difference in the serum cholesterol level of 3-year females (403 mg/100 cc) and 3-year males (333 mg/100 cc) but no differences between sexes in the 1- and 2-year fish. Age appears to be a factor since 2-year-old males had higher levels than 3-year-old males (382 mg/100 cc vs. 333 mg/100 cc) and 3-year-old females had higher levels than yearling females (403 vs. 358).

ix - Determination of seasonal changes of blood cholesterol of male and female brown trout

At the end of the first 6 months of a 1-year experimental period, there are seasonal differences in blood cholesterol of male and female brown trout.

During the months of July, October, November, and December, female trout blood serum was significantly higher in cholesterol than that of male trout (222, 236, 295, and 246 vs. 154, 156, 203, and 162). Although female blood cholesterol was apparently higher than that of males in August and September (445 and 101 vs. 397 and 90) these differences are not significant. There are differences within sexes between months. The highest levels were reached for both sexes in August (445 mg/100 cc blood serum for females and 397 for males) and lowest levels in September (101 for females and 90 for males). In October blood serum cholesterol increased to 236 mg/100 cc for females and 156 for males and have remained near these values during November and December.

Determination of the effect of diet and water temperature on blood cholesterol of brown trout fingerlings

Brown trout fingerlings were held at two water temperatures, 8.3°C. and 11°C., for 20 weeks. Duplicate groups of fish were fed either a meat-dry meal diet or a meat-dry meal diet supplemented with poultry A and D oil, cottonseed oil, or hydrogenated cottonseed oil. At the end of 10 and 20 weeks blood serum of the fish was analyzed for total serum cholesterol and at the end of 20 weeks for total serum lipid.

Statistical analyses of data indicate that diet, water temperature, and time significantly affected total serum cholesterol. At the end of 10 weeks average values were 40 percent higher than at the end of 20 weeks; at 8.3°C. average values were 40 percent higher than at 11°C.; and average values of fish fed poultry A and D oil were 50, 42, and 40 percent higher than those of the fish fed control, hydrogenated cottonseed oil or cottonseed oil diets, respectively.

Diet and water temperature altered total lipids of blood serum. At 8.3°C. average values were 28 percent higher than at 11°C. The fish fed cottonseed oil had total serum lipids of 90, 87, and 75 percent higher than that of fish fed poultry A-D oil, the control diet, or hydrogenated cottonseed oil, respectively.

Effect of dietary protein on serum protein of brook trout

Blood of fingerling brook trout was analyzed to determine the effect of dietary protein upon the total proteins, albumin, and globulin of the serum after a 20-week experimental period. Diets were composed of 50 percent meat (spleen and beef liver) and 50 percent of one of three dry meal mixtures, the first containing fish meal as a major source of protein, the second meat meal, and the third soybean meal.

Fish fed diet containing fish meal showed higher levels of serum protein than did serum of fish fed diets containing either meat meal or soybean meal. Changes in total protein was reflected more in changes of serum albumin than in changes of globulin.

Effect of sex and reproductive stage on hemoglobin of brown trout blood

Three-year-old brown trout were sampled periodically to further study the effect of fish sex and their reproductive stage on the hemoglobin values of their blood.

In late June 1964 the hemoglobin values for males and females were similar (8.37 grams per 100 milliliters). By the middle of August male fish had higher average values than did the females (8.31 vs. 6.94 grams per 100 milliliters of blood).

Differentiation of hemoglobin values, by sexes and season was still evident at the last sampling date in mid-November. The studies are being continued.

VITAMIN REQUIREMENTS OF TROUT

Effects of feeding vitamin E deficient diets to brook trout

Fingerling brown trout were fed a diet containing 165 parts per million of d-alpha-tocopherol acetate for 16 weeks. Changes in microhematocrit values, body weight, and mortality rates of the fish were compared to those of brown trout fed an E deficient diet.

From the 8th through the 12th week more than an eightfold increase in mortality occurred in the tocopherol-deficient fish. By the end of the 10th week, the mean microhematocrit of the tocopherol-deficient fish had declined to 58.6 percent of that for fish receiving tocopherol.

There was no significant difference in growth rates between the two groups of fish.

One-half the remaining tocopherol-deficient fish were placed on a recovery diet (containing about 370 parts per million of tocopherol) at the end of 13 weeks. The mortality was negligible within 2 days. Total mortality for the last 3 weeks of the study was 22 and 82 percent for the recovery and tocopherol deficient groups, respectively.

At the end of the experiment average microhematocrit for the recovery group was similar to that of the control (33.3 vs. 34.5 percent) while that of the tocopherol-deficient fish was only 17.3 percent.

Toxicity of the chemical form of vitamin A for trout

Vitamin A as a palmitate or as an acetate was added to the diet of brook trout at low levels (5,500 U.S.P. units per pound of diet) and high levels (550,000 units per pound) for a 28-week experimental period.

There were no indications of toxicity for the acetate form of the vitamin at either low or high levels or at low levels of the palmitate, but there were indications of a toxicity for the high level of vitamin A palmitate as shown by a reduction in fish growth. The low level of vitamin

A palmitate and both the low and high levels of vitamin A acetate had a stimulatory effect on trout growth.

There was no evidence of increased fish mortalities.

Effect of spawning activity on the pyridoxine requirement of trout

Earlier indications that the pre-spawning period and the onset of spawning prolonged the appearance of a pyridoxine deficiency in adult brown trout appeared to be confirmed by the present series of experiments. Although the experiments are still in progress, they have to date duplicated previous observations.

EFFECT OF PHYSICAL FACTORS ON GROWTH OF HATCHERY TROUT

Comparison of total weights gained by small and large trout from equal starting weights

A group of brook trout was separated into two size groups designated large and small. Triplicate troughs of equal weights of each size group were established and held in constant temperature water. At the end of 26 weeks there is no difference in the gain in weight between the two size groups indicating that there is no advantage for a hatchery to retain one relative size group over another since the total weight gained by small and large fish from a single population were equal.

Evaluation of Haskell's estimate of increase in fish length

To evaluate the postulate that trout increase in length at a rate constant for each species when held in a constant water temperature, the length of fish used in the above experiment was estimated by establishing the number per pound from the total weight and numbers and then referring to length-weight relationship tables. The first 3 months the trout grew at a reasonably constant increase in length (approximately 0.5 inches per month). The latter part of October, the month of November and the early part of December, the increase in length tended to decrease. Since

the fish were held in water at a constant temperature (8.3°C.), temperature is not a factor in these results.

Retention of stamina by brook trout

A stamina tunnel has been constructed following the general plan of the one used by Roger Burrows although it is smaller in size. The tunnel is an aluminum pipe 6 feet long and 6 inches in diameter, supplied with water from a 5-inch propeller type pump. The flow is controlled by decreasing or increasing the by-pass water with a sliding gate operated by a small electric motor and a mechanical screw jack. The velocity can be smoothly regulated from 0.9 to 2.5 feet per second.

In May, groups of brook trout were established on the basis of their ability to withstand a current in the stamina tunnel by subjecting 12 samples of 200 fish each to the same conditions in the tunnel. The first 50 of each sample to emerge was retained and designated as weak, the next 100 discarded, and the last 50 retained and designated as strong. By this method three groups of weak and three groups of strong, each containing 200 fish were obtained and maintained in separate troughs. The fish were re-tested three times during the 22-week experimental period.

At the end of 10 weeks in water at a constant temperature of 7.7°C. the strong trout yielded performance indexes significantly higher than the weak trout. At the end of 18 weeks in water varying from 9.4 to 11.7°C. during the test, the strong trout gave performance indexes significantly greater than that of the weak after the data were subjected to analyses of covariance in which water temperature was an independent variable. At the end of 22 weeks in water varying from 4.4 to 5°C. there were no differences in the performance indexes of the strong and the weak trout. It seems probable that at the lower temperature of the third test, trout activity was depressed sufficiently so that our methods were too insensitive to detect differences. Separation by relative stamina appeared to be enduring for the first 18 weeks.

Effect of tagging method on trout growth and tag retention

Three groups of trout were tagged by 3 methods and after recording the individual weight and length of each trout, were held in separate troughs supplied with water at a constant temperature of 8.3°C. At the end of the first 24 weeks there was no difference in the growth rates, mortalities, or retention of the tags. A group of untagged trout produced similar results. The barb-pennant type of tag has caused crooked bodies in some of the fish indicating improper tag application. The trout were 3 inches in length at the time of tagging and some of these small trout were inadvertently harmed when the barb was inserted. Any of the tagging methods would be suitable for future studies.

MINERAL METABOLISM OF TROUT

Calcium absorption by developing brown trout sac fry

Brown trout eggs and sac fry were exposed for six 24-hour periods to calcium-45 labeled waters (calcium concentrations of either 5, 50, or 500 ppm) during the period from one week prior to complete hatching until the yolk sac was nearly absorbed.

The greater recoveries of labeled calcium in the trout tissue were measured at 7 days before and 24 days after hatching. A minimum absorption of dissolved calcium occurred during the first 24 hours of their existence as fry. During this period the brown trout also contained the least amount of total calcium. The fry markedly increased their calcium contents and rates of calcium uptake as they developed.

The fry held in the 500 ppm calcium concentration absorbed and contained the most calcium. Prior to hatching, however, the eggs in the normal 50 ppm calcium concentration of Cortland water absorbed and contained the most.

The fry in the 5 ppm calcium water increased the rate of calcium absorption the fastest

As they developed, until at 24 days after hatching their daily calcium uptake nearly equalled that of the fry in the higher level calcium waters; the calcium content of the fry in the soft water had increased by about 50 percent, and their rate of calcium exchange was about 10 times greater than it had been during the first day after hatching.

By the time the yolk-sac was nearly absorbed, a well regulated calcium exchange was maintained by the fry in all three calcium concentrations.

Effect of environmental stresses on the regulation of dissolved calcium absorption by brown trout fingerlings

Brown trout were transferred from the normal 50 ppm calcium concentration of our water to synthetic waters of either 1 or 50 ppm calcium and that contained either 110 ppm strontium (as SrCl_2) or 5,000 ppm chloride (as NaCl), or both strontium and chloride or either of them. Along with these chemical changes the fish were stressed with abrupt temperature changes (from 9°C . to either 10.5°C ., 10.5°C ., or 19°C .). These synthetic waters were labeled with Ca^{45} .

The recoveries of the labeled calcium in the skeletal and muscular tissues showed that at the 50 ppm calcium concentration more calcium exchange occurred in the presence of the high concentration of sodium chloride and less occurred in the presence of the strontium ion. All of the fish held in the higher calcium waters survived the stress of transferral to the different environment.

The fish held in the 1 ppm calcium concentration at the two colder water temperatures in the 110 ppm strontium (equimolar to 50 ppm calcium) also survived all the stresses. Mortalities of the fish generally occurred with combinations of warmer water, low calcium, strontium, and high salinity, and can be related with an increased demand for dissolved calcium precipitated by increased metabolism of the fish, by an antagonistic effect of sodium chloride, and by the absence of the supply of another divalent cation.

In the higher calcium waters, complete turnovers of the trout's tissue calcium occurred in the 19°C . water after only 4 days in the labeled water. This could not occur with the limited supply of calcium in the soft water, and the presence of the divalent strontium ion did not prevent mortalities of the fish after three days in the low calcium water at 19°C .

About one-third of the exchanged calcium was recovered in the skeleton of the trout, regardless of the demands placed upon the fish by the stresses of the environment.

Distribution of dietary calcium to the skeleton and skin of brown trout

Fingerling brown trout were fed encapsulated synthetic diets that contained either of two levels of calcium-45 labeled calcium (0.5 or 2.0 milligrams), and either no phosphorus or phosphorus at a one to one ratio with the calcium. From the low and high levels of calcium without any phosphorus, respectively, 3.5 and 2.5 milligrams of dietary calcium were converted to one milligram of labeled calcium in the tissues after 2 days. The conversions were about three times poorer when dietary phosphorus was present to chemically bind some of the calcium in the encapsulated foods. The presence of dietary phosphorus did not increase the distribution of calcium to the skeletons of these fish; the bone calcium exchange proceeded efficiently in the absence of a concurrent source of phosphorus over a 4-day period after feeding.

With all four rations, about one-fourth of the dietary calcium was recovered in the skeleton, and about one-half was recovered in the skin. The skin of the brown trout contained nearly one-half of its total calcium also, and the skin appeared to function as a calcium storehouse. Similar recoveries of dietary phosphorus in the skin of brook trout have been made previously at this laboratory.

Distribution of labeled calcium to the skeleton and skin occurred within one day after feeding. The calcium of the muscle tissues, while relatively low in total amount, contained proportionately more labeled calcium, primarily that transported in the fluids included in the

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muscle sample. These muscular tissues, however, retained only a small portion of the ingested calcium 4 days after feeding. Both the

skeleton and the skin of the brown trout retained a significant portion of the dietary calcium at this time.

STAFF

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Differences in protein requirements were measured as skeletal growth increased in age. Quantitative tryptophan requirement for brook, coho, and sockeye salmon was found constant at 0.25 percent of the diet.

Nitrogen wastes from fish fed intermediate in the digestive cycle were increased. D-amino acid oxidase activity and specificity was found in chinook salmon liver and kidney tissue.

Pathways of carbohydrate metabolism showed the first step of the pentose cycle present. The second step was less distinct suggesting increased sensitivity or an alternate route for sugar metabolism.

Erythrocyte, liver, and muscle transaminase activity was directly related to tolerance whereas transaminase activity was correspondingly related to pyridoxine deficiency. Other data suggested active carbohydrate metabolism within the erythrocyte.

The amino acid patterns in salmon viscera and metabolic organs were assembled and the biological nitrogen balance was related with new and additional tissues.

Cooperative studies investigated phytoestrogen and histrogen in blood clotting mechanisms, studied chemical feedback mechanisms in liver with and without tumors, examined carcinogen intermediates in the urine and blood, and brought an electron microscope to bear on changes in normal, toxic, and tumorous livers.

Adult brook trout fed a diet for three salmon was retarded, however the effect by protein was not in water. Growth and development were delayed and white spots appeared.

Di-methyl sulfoxide (DMSO) had little effect on growth and survival and apparently increased cell permeability for nutrients and chemicals. High level showed degeneration of some tissues, high hemoglobin and depression of erythrocytes.

Histopathology confirmed renal failure of hepatoma tumor during the past 3 years as described on a page of abstracts.

NUTRITION AND BIOCHEMISTRY

Measurements of protein quality

Past studies with fish demonstrated that protein efficiency ratio (PER) varied with tissue. A dietary level of approximately 20 percent protein (percent of wet diet) produced nearly maximal PER values when a variety of widely differing protein sources were fed to coho salmon. It was therefore, possible to formulate standard rations in which most protein sources, although widely differing in protein content, could be tested in relatively isocaloric diets.

PER values established under these conditions were shown to be reproducible and in a series of assays to a degree of precision comparable with those obtained in rat studies. It was also found that although PER varied with dietary level of protein, rate of feeding fed fish

WESTERN FISH NUTRITION LABORATORY

Cook, Washington
John E. Halver, Director

HIGHLIGHTS

Improvements in measurement of protein quality showed positive correlations between protein efficiency ratio (PER), net protein retention (NPR) and nutritional response. First and second limiting amino acids were determined for two standard protein sources and complementary effects of protein ingredients were demonstrated.

Differences in protein requirements were measured as steelhead trout increased in size. Quantitative tryptophan requirement for chinook, coho, and sockeye salmon was found consistent at 0.25 percent of the diet.

Nitrogen wastes from fish fed intermediates in the citrulline cycle were measured. D-amino acid oxidase activity and specificity was found in chinook salmon liver and kidney tissues.

Pathways of carbohydrate metabolism showed the first step of the pentose cycle present. The second step was less discrete suggesting increased sensitivity or an alternate route for sugar metabolism.

Erythrocyte, liver and muscle transketolase activity was directly related to thiamin status whereas transaminase activity was correspondingly related to pyridoxine deficiency. Other data suggested active carbohydrate metabolism within the erythrocyte.

The amino acid patterns in salmon tissues and metabolic organs were assembled and the biological tissue bank was replaced with new and additional tissues.

Cooperative studies fingerprinted plasminogen and fibrinogen in blood clotting mechanisms, studied cholesterol feedback mechanisms in liver with and without tumors, examined carcinogen intermediates in the urine and blood, and brought an electron microscope to bear on changes in normal, toxic, and tumorous livers.

Cottonseed meal fats, prepared by USDA and NCPA, were found positive for hepatoma induction by feeding. High load aflatoxin tests were lethal, and chronic ingestion of low levels resulted in primary liver cancer; thus a cause for trout hepatoma was defined.

Iodide uptake was shown not to be a true measure of thyroid activity in chinook salmon.

Acute copper and zinc toxicity for three salmon species was recorded; tolerance increased by pretreatment with zinc in water. Copper and zinc changed embryonic development and white spot appeared.

Di-methyl sulfoxide (DMSO) had low toxicity to trout and salmon and apparently increased cell permeability for nutrients and chemicals. High level showed dehydration of some tissues, high hematocrit and degeneration of erythrocytes.

Histopathology confirmed visual indices of hepatoma tests during the past 3 years and described toxicity of aflatoxin.

NUTRITION AND BIOCHEMISTRY

Measurements of protein quality

Past studies with fish demonstrated that protein efficiency ratio (PER) varies with intake. A dietary level of approximately 20 percent protein (percent of wet diet) produced nearly maximal PER values when a variety of widely differing protein sources were fed to coho salmon. It was therefore, possible to formulate standard rations in which most protein sources, although widely differing in protein content, could be tested in relatively isocaloric diets.

PER values established under these conditions were shown to be reproducible and in a series of assays to a degree of precision comparable with those obtained in rat studies. It was also found that although PER varies with dietary level of protein, rate of feeding (ad lib

versus 2/3 ad libitum rate) had little effect on PER values in two trials with casein as the sole source of protein. Although maximum PER values may vary with protein sources, generally a single dietary level may be employed with fish to measure relative protein quality.

The second criticism of PER is that it does not consider body composition. Fat deposition, particularly, may be influenced by numerous factors including nutrition, species, individual, season, and environment. Rather wide variations in body composition were encountered; however, a positive correlation was found between percent of ingested protein retained by the animal and the PER (coefficients of correlation 0.93-0.98).

These experiments showed that relative protein quality for fish can be measured by feeding trials under standardized conditions with a degree of precision comparable to similar studies with other animals. Weight gain, protein utilization, diet efficiency, net protein retention or protein efficiency, can be calculated from the data.

Limiting amino acids of two protein sources

Respective protein sources were supplemented with triads of amino acids. Methionine was indicated as limiting in casein whereas tryptophan was indicated as limiting in cottonseed meal. Supplements of single amino acids at 0.5 or 1.0 percent of the dry diet were investigated in a second trial. Methionine and arginine were limiting in casein, confirming the first observations. However, in cottonseed meal diets phenylalanine and leucine supplements produced increased weight gains.

A frequent difficulty encountered in studies of limiting amino acids is the creation of an actual amino acid imbalance by excessive supplementation of what may actually be the limiting amino acid. Imbalances may have been produced in some of the previous trials, particularly in those using cottonseed meal.

A third study was completed in which the limiting amino acid was predicted from a graphical plot of the amino acid spectrum of the protein

in relation to the amino acid requirements of the animal. By this method arginine was indicated as the first limiting amino acid of casein and methionine-cystine as the second. Cottonseed meal (CSM) appeared almost equally limiting in tryptophan and lysine. To test the prediction 20 percent casein diets were supplemented with 0.15, 0.30, and 0.60 percent arginine; with 0.10, 0.20, and 0.40 percent cystine; and with combinations of the respective levels of the test amino acids.

CSM diets were similarly treated except that levels of 0.025, 0.050, 0.10 gms/100 gms dry diet of L-tryptophan and 0.16, 0.32 and 0.64 gms of L-lysine were added. Again, three combinations of the two amino acids were fed. Results were inconclusive due to variation in rates of feeding, particularly the CSM supplemented diets. Combination of 0.05 gms L-tryptophan and 0.32 gms L-lysine showed the greatest weight gain and highest PER. Diets supplemented with single levels of one amino acid did not produce growth greater than the controls. All but one of the supplemented casein diets out-performed the controls with arginine supplemented diets showing the highest rate of gain. L-arginine, 0.15 gms, produced gain equal to levels afforded by 0.30 and 0.60 gms. Similar results have been obtained in other animal experiments indicating the supplemental level of a limiting amino acid to be quite critical.

It appeared that cystine (very low in casein) may partially satisfy the requirement for methionine because cystine supplemented diets out-performed the controls. Maximum gain and PER were obtained at the 0.20 gm level. Combinations of arginine and cystine did not improve the dietary quality more than supplementation with arginine alone. The gain at the lowest level of arginine and cystine equalled that of the higher levels, again pointing out that a minimum level of a supplementary limiting amino acid is required to enhance dietary quality.

A feeding trial designed to measure supplementary action of protein to a reference protein was concluded. Casein was taken for the reference protein and compounded into diets at levels of 30, 35, 40 and 45 percent (N x 6.25). Test diets were compounded which contained 35

percent casein plus 5 percent of the test protein. All diets were calculated isocaloric and those containing test protein maintained isonitrogenous with the 40 percent protein level control. All diets were fed at the same protein level (dry weight) and at a rate of approximately 4 percent (wet weight) of the body weight per day to 100 coho salmon for 12 weeks. Results of the trial are summarized graphically in figure 1. Increase in weight gain was nearly linear in control diets. Considering weight difference between 35 and 40 percent levels of casein as 100, then cottonseed meal was only 75 percent as effective as casein. Gelatin was 110 percent as effective, fish meal, skim milk, poultry by-product meal and feather meal were 282, 307, 325, and 370 percent as effective, respectively. The above method of scoring may be inexact, but the supplementary quality of the various protein sources was dramatically illustrated. Proximate analysis data have not been compiled for this experiment; it is expected, however, that the weight gain should reflect protein deposited. Increased PERs were also obtained and in previous studies high coefficients of correlation ($r=97+$) have been demonstrated between PER and percent protein deposited.

The above procedure might be employed as a bioassay of individual ingredients. When particularly promising combinations of proteins are found, further experiments to determine optimal ratios would be in order. Entire rations might be "built" in this manner by using combinations of protein sources as the reference protein and substituting the third test protein. The experiment does not imply that any of the proteins tested would necessarily be good major sources of protein; it merely suggests these materials should be considered as protein supplements.

D-amino acid oxidases in trout and salmon

D-amino acid oxidase activity has been detected and measured in rainbow trout and chinook salmon (liver and kidney). For general activity, a sensitive colorimetric assay method utilizing the formation of a colored compound from D-allohydroxyproline was adapted for use with fish tissues. Activity was very susceptible to inactivation during storage of extracts and prepared tissue material (including lyophilized preparations). The best stability was obtained with fresh or freshly frozen tissue.

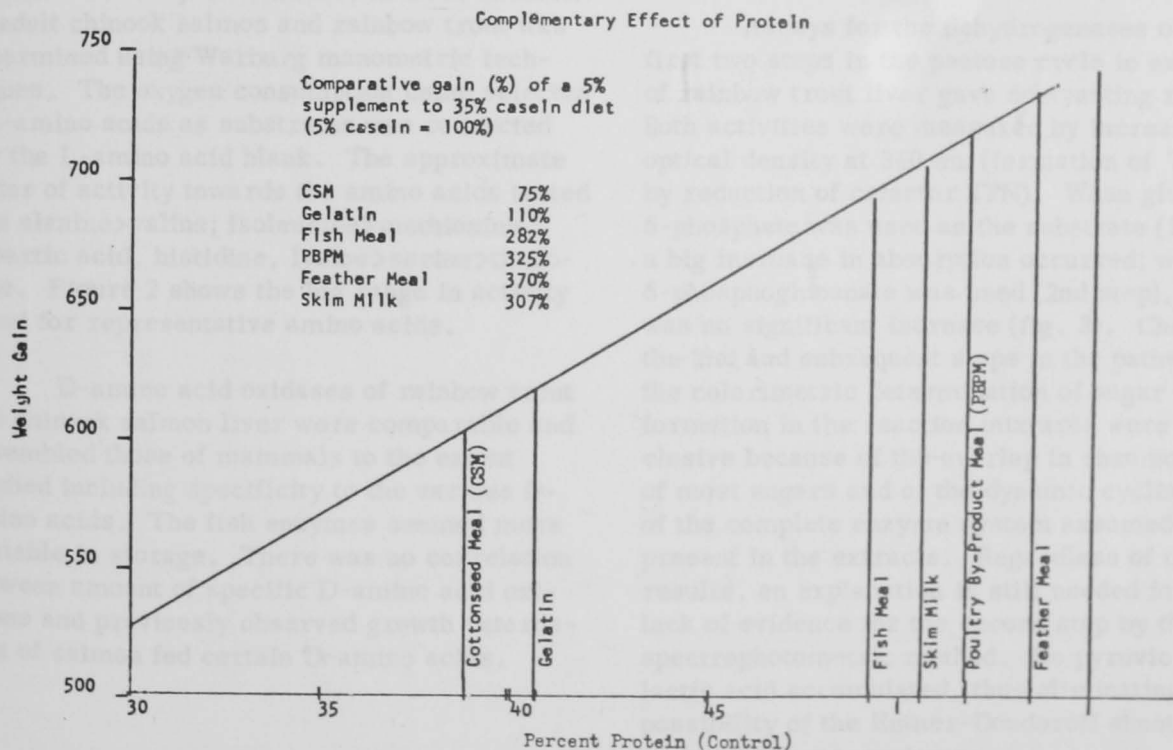


Figure 1:--Classification of complementary effect of some fish diet proteins.

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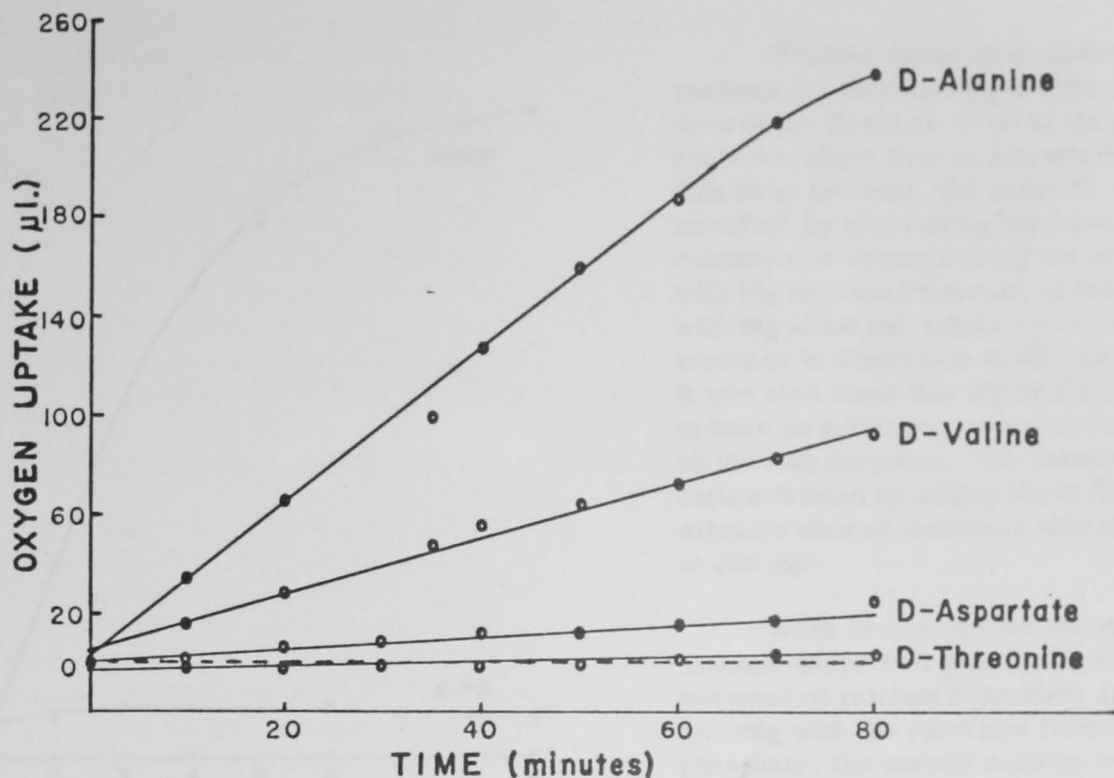


Figure 2:--D-amino acid oxidase activities of extract of chinook salmon liver. Oxygen consumption with DL-mixture of each amino acid corrected for L-blank.

The relative amount of activity for catalysis of the oxidation of selected individual D amino acids by the oxidases in liver extracts of adult chinook salmon and rainbow trout was determined using Warburg manometric techniques. The oxygen consumption using selected DL-amino acids as substrates was corrected for the L-amino acid blank. The approximate order of activity towards the amino acids tested was alanine>valine; isoleucine>methionine, aspartic acid, histidine, lysine>serine>threonine. Figure 2 shows the big range in activity found for representative amino acids.

D-amino acid oxidases of rainbow trout and chinook salmon liver were comparable and resembled those of mammals to the extent studied including specificity to the various D-amino acids. The fish enzymes seemed more unstable in storage. There was no correlation between amount of specific D-amino acid oxidases and previously observed growth retardation of salmon fed certain D-amino acids.

Pathways of carbohydrate metabolism in rainbow trout

Assays for the dehydrogenases of the first two steps in the pentose cycle in extracts of rainbow trout liver gave contrasting results. Both activities were measured by increase in optical density at 340 m μ (formation of TPNH by reduction of cofactor TPN). When glucose-6-phosphate was used as the substrate (1st step), a big increase in absorption occurred; when 6-phosphogluconate was used (2nd step), there was no significant increase (fig. 3). Checks for the 2nd and subsequent steps in the pathway by the colorimetric determination of sugar transformation in the reaction mixtures were inconclusive because of the overlap in chromogenicity of most sugars and of the dynamic cycling nature of the complete enzyme system assumed to be present in the extracts. Regardless of the latter results, an explanation is still needed for the lack of evidence for the second step by the spectrophotometric method. No pyruvic or lactic acid accumulated, thus eliminating the possibility of the Entner-Doudoroff shunt found in some microorganisms.

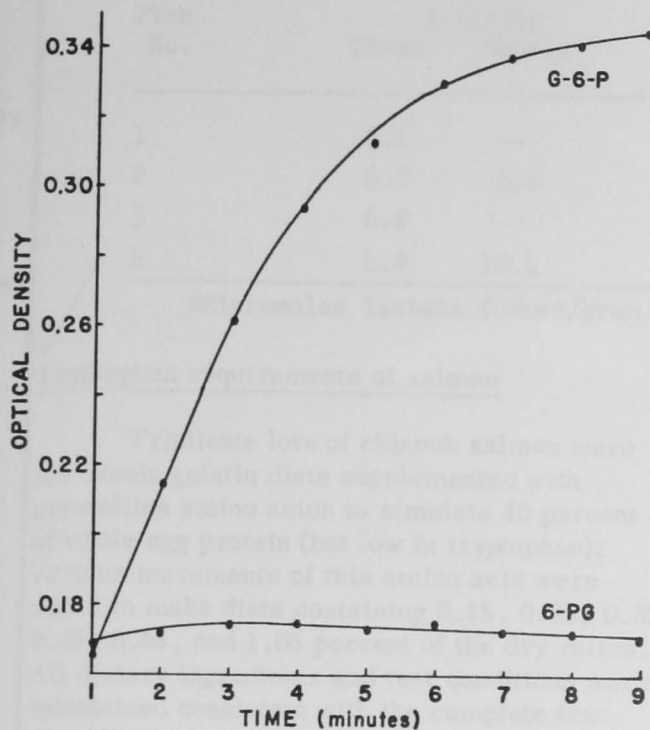


Figure 3:--Absorbance at 340 m μ showing difference in activity of extract of rainbow trout liver when treated with glucose-6-phosphate (G-6-p) and 6-phospho-gluconate (6-pG).

Regular spectrophotometric assay methods for determining the dehydrogenases of the first two steps of the pentose cycle use Mg⁺⁺ ions as activators. With fish liver extracts, the methods must be modified by eliminating Mg from the assay mixtures or by pretreating the extracts with Mg because treatment of the extract with Mg alone (no substrate) caused a big increase in absorption at 340 m μ (fig. 4). It was also found that Mg or Ca ions seemed to have no activating or stabilizing effect on the fish enzymes. The unknown substance formed by adding Mg to fish liver extracts showed maximum absorbance at 305 m μ .

Work is in progress studying the Embden-Meyerhoff glycolytic pathway enzymes of rainbow trout liver and muscle. Starting with the substrate fructose-6-phosphate, the overall activity has been determined by analyzing for the increase in lactic acid. The preliminary results in table 1 showed considerable variation in values. It was necessary to add hexokinase to the extract to obtain comparable overall activity with glucose as substrate.

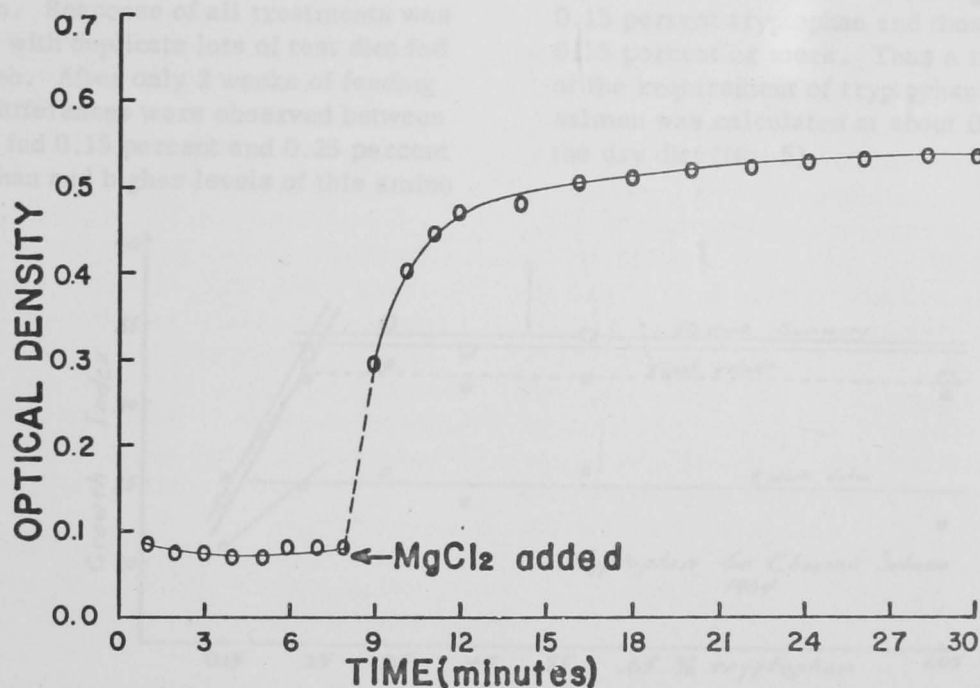


Figure 4:--Effect of magnesium ions on the absorbance at 340 m μ of an extract of rainbow trout liver.

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Table 1:--Glycolytic activity of rainbow trout liver and muscle.

Fish No.	Activity		Fish No.	Activity*	
	Liver	Muscle		Liver	Muscle
1	7.4	--	5	6.8	4.9
2	6.0	6.0	6	2.4	7.3
3	6.2	--	7	5.0	8.5
4	4.9	10.4	8	3.3	7.9

*Micromoles lactate formed/gram tissue/hour.

Tryptophan requirements of salmon

Triplicate lots of chinook salmon were fed casein gelatin diets supplemented with crystalline amino acids to simulate 40 percent of whole egg protein (but low in tryptophan); various increments of this amino acid were added to make diets containing 0.15, 0.25, 0.35, 0.45, 0.65, and 1.05 percent of the dry ration. All dietary ingredients and test conditions were maintained consistent with the complete test diet (CTD) control and those tests described previously for tryptophan requirement studies with chinook salmon fingerlings. To maintain diets isocaloric and isonitrogenous, proline was substituted for tryptophan in those treatments containing less than 1.05 percent of tryptophan. Response of all treatments was compared with duplicate lots of test diet fed control fish. After only 2 weeks of feeding discrete differences were observed between those lots fed 0.15 percent and 0.25 percent of tryptophan and higher levels of this amino

acid. Mortality was insignificant throughout the course of the experiment.

After 10 weeks of feeding, representative random samples of fish tissues were preserved for proximate analysis, for measurement of protein deposited and for future histopathological review when warranted. No significant differences could be determined between those groups of fish fed 0.25 percent or more tryptophan in the ration. In contrast those groups which received only 0.15 percent of tryptophan in the diet failed to grow as well as the control or others receiving higher levels of tryptophan in the ration. Significant differences in gain, total growth, final weight, and food conversion could be measured between the groups receiving 0.15 percent tryptophan and those receiving 0.25 percent or more. Thus a tentative estimate of the requirement of tryptophan for chinook salmon was calculated at about 0.25 percent of the dry diet (fig. 5).

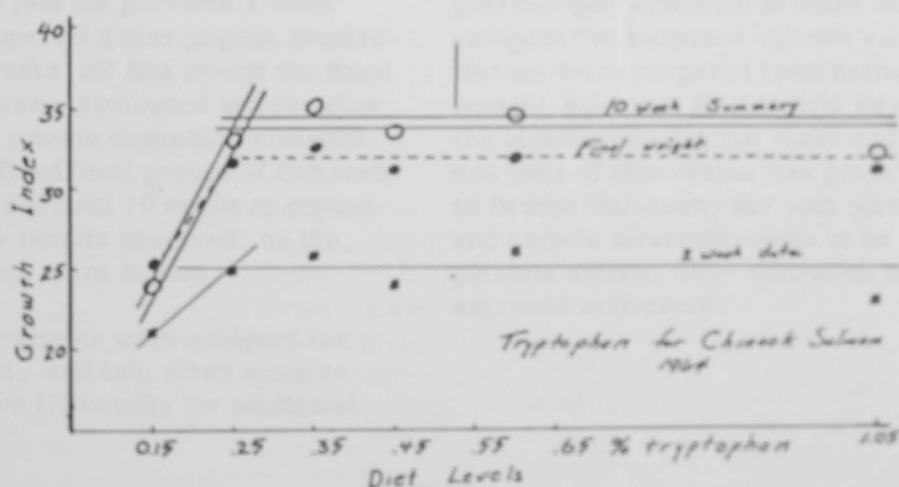


Figure 5:--Tryptophan requirement of chinook salmon

The feeding trial reported for chinook salmon was repeated in essentially the same manner for both coho and sockeye salmon fingerlings. Results were essentially the same as reported for chinook fingerlings.

Differences in tryptophan requirements under different protein intakes was not studied; therefore, it may be presumptive to equate the tryptophan requirement with a fixed percentage of the dietary protein. The conservative level of 0.25 percent of tryptophan in a 40 percent protein test diet as adequate for maximum growth of salmon appeared to be a conservative and useful level for diet formulation.

Steelhead trout protein requirements with respect to size

Triplicate lots of initial-feeding steelhead trout fry were fed high protein diets and at 2-week intervals three lots were continued at the initial protein level (55 percent) and all other groups were changed to 50 percent protein diet. In the same stepwise fashion, after each additional 2 weeks of feeding, 5 percent decrements of whole egg protein were fed to each remaining group. After 10 weeks of feeding, triplicate lots of fish had been exposed to protein decrements from 55 to 50 percent to 45 to 40 percent and 35 percent, respectively, and to all decreasing unit combinations of these protein intakes. For comparative purposes duplicate lots of fish were fed for the entire 10-week period at fixed levels of simulated whole egg protein, at 35 percent, 45 percent, and 50 percent, respectively. Results from these two sections of experiments were compared with duplicate lots fed a floating protein level, calculated from the previous 2-week response of the apparent gross protein requirement. After 10 weeks all lots except the fixed levels of protein were terminated and samples were obtained for protein deposition measurements. The five fixed level groups of fish were continued for an additional 10 weeks to expand and to confirm the results observed as the fish increased much more in size.

Terminal samples were analyzed for water, protein, fat, and ash; other samples were sent to Purdue University for additional

analyses. Results should indicate not only the gross protein requirement of steelhead trout but also should indicate how the dietary protein requirement changes with size from the very young trout as they grow and develop in a fixed temperature environment.

Nitrogen wastes from trout

Rainbow trout were fed intermediates in the citrulline cycle. Fish held in metabolic chambers were force fed load tests of arginine, citrulline, ornithine, glutamic acid, proline, aspartic acid, asparagine, glycoyamine and glutamine, respectively. Urine was collected for 3 days after the load test and was shipped to Purdue University. Similarities and differences encountered between urinary wastes in fish and those found in other animals using this common nitrogen elimination system will be recorded following analysis during the winter quarter.

Plasminogen from salmon

Lyophilized salmon heart tissues were prepared from feeding oceanic salmon, but were not able to activate plasminogen present in oxy-lated male chinook salmon serum. Curtain paper electrophoresis assay showed a protein component where plasminogen from other animals normally separates but no clot dissolving activity was measured with this component. Plasmin-like activity was tested in the salmon and trout with inconclusive results; little reduction of blood clots could be measured. The absence of eosinophils in trout and salmon blood suggested the absence of plasmin activity could be related to the absence of eosinophils, a site of plasminogen synthesis in other animals. For comparative purposes chinook and coho salmon tissues were prepared from actively feeding oceanic salmon. Eosinophils were identified in the blood of the dogfish shark and approximately one liter of this serum was prepared and shipped to Purdue University for both plasminogen assay and curtain electrophoresis to be followed by plasmin activity after activation by common extrinsic activators.

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Replenishment of biological tissue bank

In a short intensive collection trip the bank of biological research tissue from actively feeding chinook and coho salmon was replenished. Tissues collected and prepared include lyophilized heart and liver from different class and sex of salmon; frozen heart, liver, brain, eye, gill, esophagus, stomach, caeca, colon, spleen, abdominal fat, anterior kidney, posterior kidney, skin, muscle, blood and serum. In addition three complete gastro-intestinal tracts from chinook salmon, coho salmon, rockfish, ling cod, halibut, flounder, sole, dogfish, hake, and mackerel were removed and frozen on dry ice for analysis of intestinal flora and fauna by Virginia Polytechnic Institute scientists.

Cholesterol feedback mechanism in trout

Normal and tumor bearing trout were shipped to Dr. Siperstein at the University of Texas for estimate of cholesterol feedback mechanism. In preliminary experiments load tests of normal trout with carbon¹⁴ labeled cholesterol indicated linear response in cholesterol synthesis *in situ* with respect to cholesterol injected. Further experiments are underway to elucidate the nature and the extent of this mechanism in normal and tumor bearing rainbow trout as an indicator of the extent of liver damage or liver susceptibility to abnormal stimuli.

Thyroid iodine uptakes not a true measure of thyroid function

Thyroid I¹²⁵ uptake differences between I¹³¹ treated and control chinook salmon were quite similar to those observed between thyroidectomized and control rainbow trout on identical diets. Thus, on the basis of I¹²⁷ thyroidal contents and I¹²⁵ thyroidal uptake, it would appear logical to conclude that young chinook salmon were thyroidectomized within 16 months following massive I¹³¹ treatment. However, the impressive suppression of thyroid function in the I¹³¹-treated chinook salmon could not be equated with complete functional destruction of the thyroid since subsequent chromatographic fractionation of thyroid

hydrolysates revealed the presence of monoiodo-tyrosine (MIT), diiodotyrosine (DIT) and thyroxine (T_x). It was also shown that I¹³¹ treatment induced a profound change in the intrathyroidal I¹²⁵ distribution pattern when compared to that of the controls, and vestigial thyroid function was still demonstrable. The induced change in the thyroid iodine metabolism revealed only trace amounts of the I¹²⁵ apparent in each iodoamino acid fraction of the I¹³¹-treated salmon 3 hours after injection. Over 25 percent of the I¹²⁵ in the I¹³¹-treated animals continued to be incorporated until an apparent equilibrium was established approximately 48 hours after injection. Average I¹²⁵ distributions remained nearly constant for the remainder of the time study with MIT levels ranging between 10 and 12 percent, DIT ranging between 18 and 24 percent, thyroxine between 14 and 17 percent and iodide concentration between 45 and 57 percent of the thyroid region iodine. Conversely the I¹²⁵ distribution in control fish exhibited two principal differences, while MIT and DIT distribution continued to rise during the first 48 hours, the iodide level continued to decrease throughout the one-week sampling period (9 percent at 168 hours) and thyroxine levels continued to rise (14 percent at 168 hours), thus thyroid region fractionation of radioiodine labeled amino acids was necessary to establish the true level of thyroid activity in these salmonids.

Nutritional status measurements

Dr. Brin at Upstate Medical Center, Syracuse, New York, tested erythrocyte transketolase, liver transketolase, erythrocyte transaminase and liver and muscle transaminase activity of rainbow trout and salmon exposed to different nutritional treatments, at different sizes and at different ages. The analytical results showed that transaminase activity of muscle and liver was significantly depressed in pyridoxine-deficient fish. A pyridoxal phosphate effect was observed in the liver similar to the thiamin pyrophosphate effect found in thiamin-deficient fish. Erythrocyte transketolase study showed a direct relationship between the thiamin status and erythrocyte transketolase activity. Of particular importance was the consistent observation of the high pentose levels in the hemolysates (75 mg percent or about 20

times that reported in the literature for other species. Catfish blood was also examined for comparative purposes. Endogenous hexose content of catfish hemolysates was very high, (3 to 4 mg per ml). This would suggest a blood glucose level of about 600 mg percent or in excess of 6 times the physiological level for other animals, or very active synthesis of hexose by the erythrocyte. In addition, the high pentose levels were again confirmed extending the reliability of these observations and suggesting this phenomenon may be general for fishes and may again indicate active synthesis of these sugars. An alternate major pathway may be present for glucose metabolism in these animals with nucleated erythrocytes.

Carcinogen intermediates were analyzed by the Millers at McArdle

Eleven fish, contained in individual metabolic chambers, were catheterized and 30 mgs of 2-acetyl aminofluorene or 7-fluoro acetyl aminofluorene was inserted through a small incision into the body cavity. Incision was sutured and urine samples (24, 48, and 72 hours) were collected. At the termination of the experiment all fecal material was removed from the posterior end of the metabolic chamber and was concentrated for shipment to the McArdle Memorial Laboratory. These samples will be used to augment and to extend the information on absence of N-hydroxylating enzyme for 2-acetyl aminofluorene in rainbow trout.

Tests for aflatoxin were lethal

Crude aflatoxin obtained from the MIT laboratories was force fed to 10 individual 50 gm rainbow trout at 5 mg, 10 mg, 15 mg, single doses or 3 mg doses per kg/body weight per day for 5 days. All fish died by the 10th day when 15 mg/kg body weight of aflatoxin was administered. Eight of 10 fish died when 10 mg was administered, and 6 of 10 died when 5 mg/kg body weight was administered in a single dose. All fish which died showed the same symptoms. When moribund, they turned a dark color, became listless and were easily captured. Death occurred about 24 hours after external symptoms were noted. Gills became almost

white indicating severe anemia. Internally all livers were a light tan color with numerous dark red hemorrhagic areas covering 25 to 50 percent of the liver surface. The gastrointestinal tract showed moderate to severe tissue hemorrhage. Throughout the abdominal fat hemorrhagic spots were apparent. The caeca and intestines contained many red hemorrhagic areas but there were few noted in the stomach walls. No hemorrhage was noted in the musculature and the kidney appeared normal. On the basis of this study and similar load tests conducted previously, lethal dose for half the individuals (LD₅₀) for rainbow trout appears to fall between 1 and 5 mg/kg body weight for the crude material, or at less than 1 mg/kg body weight for the crystalline aflatoxins.

In another study crystalline aflatoxin B₁ obtained from the MIT laboratories was dissolved in a minimum volume of chloroform, diluted with 95 percent ethanol, added in appropriate amounts to 1 kg of alpha cellulose flour, and thoroughly blended and dried overnight to remove the solvents. Uniform distribution of the aflatoxin was checked under ultra violet light. Appropriate amounts of the concentrate premix were diluted with alpha cellulose flour and loaded into capsules to be force fed to 50 gm trout at 0.1, 0.5, 1.0, and 3.75 mg/kg body weight for single dose tests and at 0.1 and 1.0 mg/kg body weight daily for the 5-day test. Ten fish were dosed with each level of crystalline aflatoxin B₁. For comparison purposes 10 fish were dosed with the crude aflatoxin preparation at 1, 3, or 5 mg/kg body weight in a single dose and 1 mg/kg body weight daily for 5 days. On day 10 all survivors were closely examined and any internal anomalies recorded. Every fish at any treatment which survived the 10-day test had discrete, inflamed hemorrhagic areas in the liver. The extent of involvement increased with increasing insult of either the crystalline aflatoxin B₁ or the crude preparation. A tentative LD₅₀ was estimated at between 0.1 and 0.5 mg/kg of the crystalline aflatoxin B₁, and at between 3 and 5 mg/kg of liquid preparation.

Electron microscope studies of trout liver

Dr. Wellings and Dr. Phelps of University of Oregon Medical School collected and preserved livers from tumor bearing and normal trout for electron microscope studies of the intracellular architecture to compare trabecular hepatoma, parenchymal cell hyperplasia, and normal parenchymal cell structure of small, intermediate and large rainbow trout. In addition, representative samples of livers from trout challenged with crude and purified aflatoxins at chronic levels and at frank toxic levels were preserved for analysis. Some dramatic changes in intracellular liver cell architecture were observed and new sub-cellular particles were seen.

Iodide requirement studies

Rainbow and steelhead trout fed five different levels of dietary iodide failed to grow at a normal rate and soon developed a persistent mortality during each 2-week experimental period. Examination of tissues failed to locate pathogens. Vitamin A alcohol, Vitamin D₃, and corn oil were substituted for cod liver oil to reduce the iodide level of the basal ration. Replacement of cod liver oil to part of the survivors resulted in return to growth; the mortality rate dropped abruptly after 4 weeks on the new ration.

Acute copper and zinc toxicity in chinook salmon

Acute copper toxicity for chinook salmon appeared when 13 to 14 ppb copper was present in the water, regardless of age. The presence of 100 ppb zinc approximately doubled the sensitivity of the fish to copper ion. The critical age period for zinc toxicity appeared to be from hatch until about 2800 "heat units". Low concentrations of copper exhibited an apparent protective mechanism toward zinc toxicity until approximately 5,000 "heat units" when the effect appeared to change from antagonism to synergism.

Copper and zinc in embryonic development

The influence of copper-zinc on embryonic development was repeated with chinook salmon eggs from two sources in October and November. The lower limit of chronic copper toxicity was tested by exposing developing eggs and yolk sac fry to five different copper levels -- between 0.4 ppb copper present in the water supply to 5 ppb total copper in the water. The atomic absorption spectrophotometer allowed accurate and significant analysis for copper at these low levels. Concurrently eight levels of copper-zinc combinations were arranged for study of synergistic action between these two metals. Parameters under observation are: hatching rate, egg mortality, fry mortality, appearance of white spot or coagulated yolk, copper and zinc retention in the egg and fry tissue, calcium protection toward toxicity and oxygen uptake.

Hyperthyroid trout

In attempts to induce smoltification and better adaptation to high saline environments USP thyroid extract powders at 0, 2, 4, and 8 gms/100 gms dry diet were fed to 4 groups of rainbow trout. After 6 weeks of feeding part of each group were transferred to a high saline environment for one week after which all fish were sacrificed and samples of muscle and blood were saved for analyses. The degree of smoltification induced was roughly proportional to the level of thyroid powder fed (fig. 6). Although no mortalities ensued following transfer to the saline environment all fish were distressed by the transfer.

Thioacetamide and blindness in trout

Rainbow trout fed 120 mg percent of thioacetamide in the diet for 12 months became blind in one or both eyes after 9 to 12 months. Fish with varying degrees of blindness were shipped by air express to Dr. Ludwig Von Sallman, Chief, Ophthalmology and Blindness Laboratories of the National Institute of Neurological Diseases and Blindness. There, mechanisms involved in the loss of vision and specifically

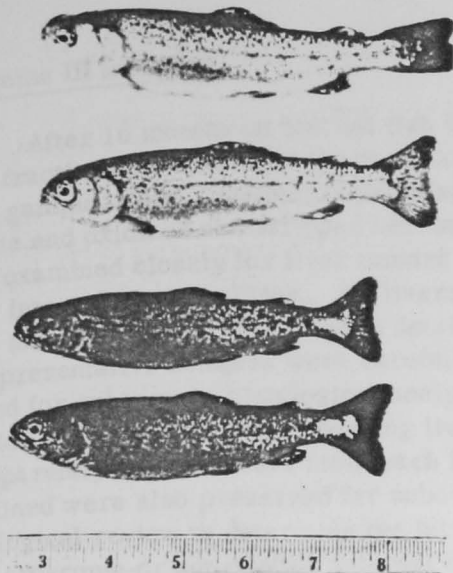


Figure 6:--Effects of feeding thyroid extract. Bottom fish is the normal rainbow trout, remaining three fish pictured were fed increasing amounts of thyroid extract, the top fish receiving the greatest amount.

those enzyme systems inhibited by thioacetamide are scheduled for study in efforts to improve understanding of degenerative atrophy of vision processes.

Fibrinogen for blood clots found different in salmon

The amino acid sequences of fibrinogen in chinook salmon blood and in lamprey eel blood were carefully fingerprinted by Dr. Jules Gladner working as a guest at the Biochemical Laboratories of Dr. Acher in Paris, France. Similarities and differences in the amino acid sequences and consequent physical chemical structure of fibrinogen from salmon and lamprey eel were compared with fibrinogen structures in the European eel, Atlantic salmon, hog, cow, chicken, and rat. The sequence of amino acids in this precursor for fibrin (the blood clot mass) in salmon blood should extend knowledge on the mechanisms of blood clotting in different species and how different, physical chemical structures have the same terminal physiological effect.

Dimethylsulfoxide tested in chinook, sockeye, and coho salmon and rainbow trout

Dimethylsulfoxide (DMSO) was tested in salmon and trout as a potential agent for increased absorption of chemicals or nutrients and the consequent increase in availability and utilization of these ingredients. This penetration of biological membranes with low order of toxicity to the cell may in addition prove to be an effective carrier for antibiotics and may enhance the metabolism and incorporation of fluorescent or other identifiable chemical markers in the growing animals. Immersion studies showed the fish could tolerate high concentrations of DMSO; the mean tolerance limit (TLM) for coho salmon was determined at 24, 48, 72, and 96 hours. A long-term study was conducted for 3 months at lower concentrations (3 percent and less). Temperature had a profound effect on the survival time of the fish, probably directly due to a change in metabolic rate. The lethal dosage for DMSO was determined by intraperitoneal injection in chinook, sockeye, coho salmon and rainbow trout, with tolerance apparently increasing in that order. Repeated dosage was used to determine cumulative effect. Oral ingestion of DMSO showed fish would accept diets containing from 1 percent up to 18 percent of the chemical; however, a loss of weight occurred above 10 percent in the diet. The apparent low toxicity at the above effective physiological concentrations for increased permeability of biological membranes should herald DMSO as an important potential research or therapeutic compound in fish husbandry.

Amino acid patterns in salmon organs

Fourteen of the main body organs of spawning chinook salmon were analyzed for moisture, ash, lipid, protein and amino acid patterns. Amino acid concentrations in brain, eye, gill filament, atrium, ventricle and bulbous heart, liver, spleen, testes, ovaries, anterior and posterior kidney, skin, and muscle were assayed on the Beckman amino acid analyzer and these values are available to researchers upon request.

Hepatoma III induction

After 16 months on test all fish fed aflatoxin fractions; neutral lipid fractions alpha, beta, gamma, delta, epsilon; dimethylnitrosamine and oxidized and stripped herring oils were examined closely for liver tumors or for other internal abnormalities. All livers or other anomalies were described in detail and 10 representative samples were carefully preserved for subsequent histological analysis. In addition to 10 positive tumor bearing livers, 10 apparently normal livers from each lot examined were also preserved for subsequent histological review to determine the incidence of microscopic or non-visible internal tumorigenesis in these dietary load treatments.

From a complete histological analysis with skip-serial section technique, a valid incidence of hepatoma was recorded for each group of fish fed each carcinogen. Examination of approximately 2,000 individual fish showed a

variation in the incidence of tumors which could be generally correlated with the chronic level of ingestion of the particular chemical compound loaded in the diet. The incidence of tumors encountered in neutral lipid fractions was low and many of the small tumors did not appear to have developed greatly since the surgical inspection at 12 months. At the 12-month inspection period, 10 tumor bearing livers had been removed from each lot and perhaps as a result most of the rapidly growing tumors present had been previously selected for histological review. In the event subsurface or new tumors might appear between the 16-month and 20-month period these groups of fish fed the neutral lipid fractions were closed surgically and returned to the tanks for an additional 4 months on assay. In general, the results paralleled the one year surgical inspection. A brief summary of the dietary treatments with those livers classified as tumor bearing, suspect or normal can be found in table 2.

Table 2:--Tumor incidence in rainbow trout following 16-month bioassay of suspect carcinogens.

Lot #	Diet	Total	Normal	Suspect	Tumors
137	B ₁ 2 ppb	64	53	6	5
138	B ₁ 8 ppb	24	20	3	1
140	G ₁ 2 ppb	55	39	11	5
141	G ₁ 8 ppb	8	39	12	7
143	b ₁ 20 ppb	62	7	9	46
144	b ₁ 80 ppb	64	5	13	46
146	g ₁ 20 ppb	74	72	1	1
147	g ₁ 80 ppb	55	42	13	
152-3	Crude 320 ppb	119	59	9	51
154-5	Crude 80 ppb	105	23	4	78
156-7	Crude 20 ppb	109	73	12	24
186-7	C.T.D.	50	49		1
172-3	O.H.O. 6%	88	76	7	5
174-5	S.H.O. 6%	93	84	5	4
170-1	DMN- 30 mg%	89	74	4	11
128-9	Total fat 6%	51	17	13	21
131	Neutral Lipid	48	30	17	1
132-3	Pellets	54	17	8	29
176-7	Alpha Fraction	47	31	15	6
178-9	Beta Fraction	39	32	7	
180-1	Gamma Fraction	46	40	6	
182-3	Delta Fraction	49	42	6	1
184-5	Epsilon Fraction	49	47	1	1

Hepatoma IV induction experiments

Samples from each of the neutral lipid subfraction fed groups of fish disclosed a high visible index of small nodules in the alpha fraction fed group and no tumors were observed in the beta, gamma, or epsilon fed fish. Two small tumors were observed in the delta fraction fed fish. When 30 to 40 livers from each group were carefully analyzed histologically it was apparent that numerous early tumors were present in livers of the alpha fraction fed fish. The alpha fraction was therefore subjected to intensive study and fractionation on pilot columns into groups of components which could be bioassayed. Three discrete families of compounds were separated using ratios of normal hexane and diethyl ether. Alpha fraction I disclosed a non-fluorescing oil which was Lieberman-Burchard positive. Alpha fraction II, the major peak, contained a light yellow oil which fluoresced with a blue-green color and which was also Lieberman-Burchard positive. Alpha fraction III was a solid and fluoresced with intense blue-white color. The same three fractions were obtained with appropriate quantities of eluant in the large chromatography columns. These were concentrated and incorporated into the the hepatoma IV feeding experiments at that level found in the original suspect feed. Other groups of fish were started in February and were continued to compare with those on other treatments at other stations from the same egg stock. Those groups which had been fed the alpha, beta, gamma, delta, and epsilon fractions for 8 weeks were changed to complete test diet and all bioassay material reserved for positive control studies when the alpha subfractions were later started in April with Winthrop stock eggs.

Bioassays of cottonseed meal fats, polymerized fats, crystalline and crude aflatoxins, were sampled at 5 months on test. Only one or two small nodules were seen on close visual examination of 10 fish from each duplicate lot on each dietary treatment. Histological review, however, disclosed an incidence of over half of micronodules in one group of fish fed cottonseed meal fat #2. Small nodules of typical trabecular rainbow trout hepatoma were seen scattered throughout the liver tissue of these

small trout. A request for aflatoxin assay through the National Cotton Producers Association resulted in a report of considerable aflatoxin B₁ present in the original cottonseed meal from which this fat had been extracted. Other cottonseed meal fat fed groups had a low or negative incidence of tumors at the 5-month sampling period and also a low or negative chemical assay report on aflatoxin load in the original cottonseed meal. Histological screening of livers of crude aflatoxin fed fish in the same experiment disclosed these positive controls had an incidence of micronodules of the same order of magnitude as that observed in the cottonseed meal fat #2 fed groups of fish. On the basis of these positive results, and corresponding parallel negative results with negative controls, and with those lots receiving equivalent cottonseed meal fat but with low aflatoxin assay levels in the original material, aflatoxin B₁ would be indicated as cause for hepatomagenesis in rainbow trout fed this cottonseed meal. It is anticipated further critical confirmatory data will be obtained as the bioassay is continued and a much more thorough examination of all fish exposed to these various dietary treatments can be completed (scheduled February 1, 1965). At the present state of the experiment, however, chemical assays of the aflatoxin load do support the carcinogenic response observed in these rainbow trout. Table 3 presents the parameters under investigation in these experiments.

HISTOPATHOLOGY

Induction of hepatoma in rainbow trout

Livers from induction experiments II, III, and IV-A were skip serial sectioned, stained with hemotoxylin and eosin and the incidence of trabecular hepatoma in each selected liver was recorded. Grossly normal and grossly suspect livers appear as separate hepatoma incidences in tables 4, 5, and 6.

The numerator of each tabulated fraction represents the number of livers which showed classical trabecular hepatoma while the denominator represents the total number of livers in the sample. Cholangioma or marked cholangiolar

Table 3:--Hepatoma IV and IV A induction experiments.

Hepatoma IV

Trial initiated 3 Feb 64
Ennis stock rainbow

Lot Number	Diet Number	Diet Treatment
192 & 193	131	1% Phospho Lipid
204 & 205	193	CSM #1 0.29%
206 & 207	194	CSM #2 0.69%
208 & 209	195	CSM #3 0.66%
210 & 211	196	CSM #4 0.44%
212 & 213	197	CSM #5 0.73%
214 & 215	198	CFP 0.675%
216 & 217	199	CFP .25%
218 & 219	200	CFP 1.0%
220 & 221	201	CFP .25% plus AAF 15 mg%
222 & 223	202	CFP .25% plus DMN 15 mg%
224 & 225	203	Polymerized fat 0.25%
226 & 227	204	DMN 15 mg%
228 & 229	62	AAF 15 mg% (1/4X)
230 & 231	205	N-OH-AAF 3 mg%
232	118	CTD
234 & 235	136	Thioacetamide 30 mg%
236 & 237	154	Aflatoxin B ₁ 0.5 ppb
238 & 239	155	Aflatoxin B ₁ 2 ppb
240 & 241	156	Aflatoxin B ₁ 8 ppb
242 & 243	157	Aflatoxin G ₁ 0.5 ppb
244 & 245	158	Aflatoxin G ₁ 2 ppb
246 & 247	159	Aflatoxin G ₁ 8 ppb
248 & 249	170	Crude Aflatoxin 20 ppb

Hepatoma IV A

Trial initiated 4 May 64
Winthrop Stock rainbow fry

253	212	Alpha I 0.1%
254	213	Alpha II 1.4%
255	214	Alpha III 0.7%
256	179	Alpha 2.6%
257	180	Beta 0.9%
258	181	Gamma 0.6%
259	182	Delta 0.8%
260	183	Epsilon 0.2%
261	156	B ₁ 8 ppb
262	159	G ₁ 8 ppb
263	215	B ₁ G ₁ 8 ppb each
264	216	Crude 40 ppb
265	118	Control

proliferations are indicated in the tables by a "ch" preceded by the number of "ch" livers in the sample; (found in aminoazotoluene and thioacetamide diets). High incidences of classical hepatoma were found in liver samples from fish fed dimethylnitrosamine, fat and carbohydrate fractions of the commercial pellets, and whole pellets (table 4).

In table 5 the aflatoxins and dimethylnitrosamine plus 5 percent corn oil showed high hepatoma incidences. High incidences for aflatoxin diets sampled after only 6 months are shown in table 6 which also shows a pronounced incidence of hepatoma for one of five cottonseed meal fat diets which assayed 300 ppb aflatoxin B₁.

Table 4:--Incidence of Hepatoma for Induction II Experiments

Carcinogen	Dose	Lot	12 MONTHS		16 MONTHS		20 MONTHS	
			N*	S*	N	S	N	S
2-Acetylaminofluorene	15 mg%	44-5					--	0/6
"	60 mg%	46-7					0/10	0/5
Aminoazotoluene	30 mg%	48-9					--	1/10 & 2 ch**
"	120 mg%	50-1					1/10	2/13 & 5 ch
"	480 mg%	52-3					0/10	-- --
Aminotriazole	480 mg%						0/10	--
Carbarsone	30 mg%	66-7					0/9	--
Dimethylnitrosamine	30 mg%	60-1	3/11	--	--	1/1	3/10	11/17
"	120 mg%	62-3	0/10	--			2/8	10/12
"	480 mg%	64-5					1/28	--
Thioacetamide	30 mg%	88-9					1/8	1/8 & 3 ch
"	120 mg%	90-1					0/11	0/11
Phospholipid	1%	74-5					& 1 ch	0/10
Fat Fraction	6%	70-1					1/5	8/9
Pellet Control	100%	80-1					4/10	5/10
Protein Fraction	25%	76-7					0/10	2/9
Carbohydrate Fraction	7%	78-9					2/12	8/10
Complete Test Diet	100%	80-1					0/13	--
Oxidized Herring Oil	6%	82-3	1/10	0/3			1/10	0/10
Active Herring Oil	6%	84-5					0/10	1/8
Stripped Herring Oil	6%	86-7					2/19	3/5

* N-- Grossly normal Livers

* S-- Grossly suspect Livers

**ch--cholangioma

Table 5:--Incidence of Hepatoma for Induction III Experiments

Carcinogen	Dose	Lot	9 months		12 Months		16 Months	
			N*	S*	N	S	N	S
Fat Fraction	6%	128-9	1/7	2/3	1/10	3/7		
Neutral Lipid	6%	131-2	0/10		1/10	1/4		
Pellet Control	100%	132-3	2/10		1/10	3/3		
Crude Aflatoxin	20 ppb	152-3						10/11
" "	80 ppb	154-5						12/13
" "	320 ppb	156-7						6/8
Aflatoxin B ₁	0.5 ppb	136	0/13**					
" "	2.0 ppb	137	0/10	0/9***			0/10	0/9
" "	8.0 ppb	138	0/10		0/11			1/4
Aflatoxin G ₁	0.5 ppb	139	0/37	Term.***				
" "	2.0 ppb	140	0/10		1/10	2/4	2/10	2/7
" "	8.0 ppb	141	0/10		1/10	0/2	1/8	2/10
Dimethylnitrosamine plus corn oil	5%	170						4/11
Oxidized Herring Oil	6%	172-3	0/10					0/10
Stripped Herring Oil	6%	174-5	0/8		0/9	1/3		2/5
Lipid Fractions: Alpha	2.6%	176-7	0/10 5/20†	0/23†	2/11	0/3		0/2 (1 tumor between caeca and liver)
Beta	0.9%	178-9	0/10		0/10	1/6		
Gamma	0.6%	180-1	0/10		1/10	1/1		
Delta	0.8%	182-3	0/9 2/39†		1/10	0/3		
Epsilon	0.2%	184-5	0/36		0/10	0/1		

* N--Grossly normal livers
S--Grossly suspect livers

** 6 months

***7 months

† 10 months

Table 5:--Incidence of Hepatoma for Induction III Experiments

Carcinogen	Dose	Lot	9 months		12 Months		16 Months	
			N*	S*	N	S	N	S
Fat Fraction	6%	128-9	1/7	2/3	1/10	3/7		
Neutral Lipid	6%	131-2	0/10		1/10	1/4		
Pellet Control	100%	132-3	2/10		1/10	3/3		
Crude Aflatoxin	20 ppb	152-3						10/11
" "	80 ppb	154-5						12/13
" "	320 ppb	156-7						6/8
Aflatoxin B ₁	0.5 ppb	136	0/13**					
" "	2.0 ppb	137	0/10	0/9***			0/10	0/9
" "	8.0 ppb	138	0/10		0/11			1/4
Aflatoxin G ₁	0.5 ppb	139	0/37	Term***				
" "	2.0 ppb	140	0/10		1/10	2/4	2/10	2/7
" "	8.0 ppb	141	0/10		1/10	0/2	1/8	2/10
Dimethylnitrosamine plus corn oil	5%	170						4/11
Oxidized Herring Oil	6%	172-3	0/10					0/10
Stripped Herring Oil	6%	174-5	0/8		0/9	1/3		2/5
Lipid Fractions: Alpha	2.6%	176-7	0/10 5/20†	0/23†	2/11	0/3		0/2 (1 tumor between caeca and liver)
Beta	0.9%	178-9	0/10		0/10	1/6		
Gamma	0.6%	180-1	0/10		1/10	1/1		
Delta	0.8%	182-3	0/9 2/39†		1/10	0/3		
Epsilon	0.2%	184-5	0/36		0/10	0/1		

* N--Grossly normal livers
S--Grossly suspect livers

** 6 months

***7 months

† 10 months

Table 6:--Incidence of Hepatoma for Induction IV and IV-A Experiments

Carcinogen	Dose	Lot	6 Months		7 Months	
			N*	S*	N	S
CFP Polymer	.0675%	214-15		0/3		
CFP "	.25%	216-17		0/2		
CFP "	1.0%	218-19		0/2		
CFP Polymer plus 2-Acetylaminofluorene	.25% 15%	220-21		0/2		
CFP Polymer plus Dimethylnitrosamine	.25% 15%	222-23		0/4		
Polymerized Fat	.25%	224-25		0/3		
Dimethylnitrosamine	15 mg %	226-27		0/1		
2-Acetylaminofluorene	15 mg %	228-29		0/2		
N-OH-Acetylaminofluorene	3 mg %	230-31		0/2		
Aflatoxin B ₁	0.5 ppb	236-37		5/7		
Aflatoxin B ₁	2 ppb	238-39		4/5		
Aflatoxin B ₁	8 ppb	240-1		9/10		
Aflatoxin G ₁	2 ppb	244	2/3	2/3		
Aflatoxin G ₁	8 ppb	246		4/5		
Crude Aflatoxin	20 ppb	248		5/5		
Phospholipid	1%	192		0/21		
Neutral Lipid Fractions:						
Alpha	2.6	194			0/2	
Beta	0.9	196			0/2	
Gamma	0.6	198			0/2	
Delta	0.8	200			0/2	
Epsilon	0.2	202			0/2	
Cottonseed Meal #1	0.29%	204	0/17	0/3	1/10	
" " #2	0.69%	206	4/10	1/2	1/10	
" " #3	0.66%	208	0/22		2/10	
" " #4	0.44%	210	1/20	0/1	0/10	
" " #5	0.73%	212	1/17	1/6	0/10	

N-- Grossly

* Normal livers

S-- Grossly suspect livers

TABLE 6 (Cont'd)

Carcinogen	Dose	Lot	4.5 Months		6 Months		7 Months	
			N	S	N	S	N	S
Induction IV-A								
Neutral Lipid Fractions								
Alpha 1	0.1%	253	0/10					
Alpha 2	1.4%	254	0/10					
Alpha 3	0.7%	255	0/10					
Alpha(Complete)	2.6%	256	1/10					
Beta	0.9%	257	0/10					
Gamma	0.6%	258	0/10					
Delta	0.8%	259	0/10					
Epsilon	0.2%	260	0/10					
Aflatoxin B ₁	8 ppb	261	0/10					
Aflatoxin G ₁	8 ppb	262	1/10					
Aflatoxin B ₁ plus G ₁	8 ppb each	263	0/10					
Crude Aflatoxin	40 ppb	264	3/10					

High load aflatoxin tests histopathology

Aflatoxin loads of 1, 3, and 5 mg/kg body weight of crude preparation were force fed to trout and livers were examined within 12 days. Toxic liver damage in the form of heavy nuclear and cytoplasmic vacuolation, pleomorphic nuclei and varying degrees of focal to extensive liver cell necrosis with marked hemorrhage were evident (fig. 7). Limited amount of cholangiolar (bile ductular) proliferation were occasionally seen. The amount of liver damage was directly proportional to the amount of crude aflatoxin ingested. Crystalline B₁ aflatoxin was similarly force fed to other trout in amounts of 0.1, 0.5, 1.0, and 3.0 mg/kg body weight with only slightly less dramatic results than those obtained by feeding the crude aflatoxin. The crystalline B₁ aflatoxin appeared to be from 5 to 10 times more potent than was the crude

preparation. Additional evidence of toxicity was seen in the form of diffuse hemorrhage in visceral adipose tissue (fig. 8) and as severe ocular edema with resultant desquamation of retinal elements (fig. 9).

Visceral tumors

Tumors of visceral organs and adnexa were noted in four different rainbow trout fed different induction diets. Each tumor resembled a lymphosarcoma and was growing attached to pyloric caeca and pancreas, stomach or liver. In one instance the tumor had invaded and partially destroyed several of the pyloric caeca. Another tumor, tentatively identified as a leiomyoma, was found growing attached to the stomach wall in a mature ocean caught coho salmon. Further work on these tumors including a more detailed description of each is contemplated.

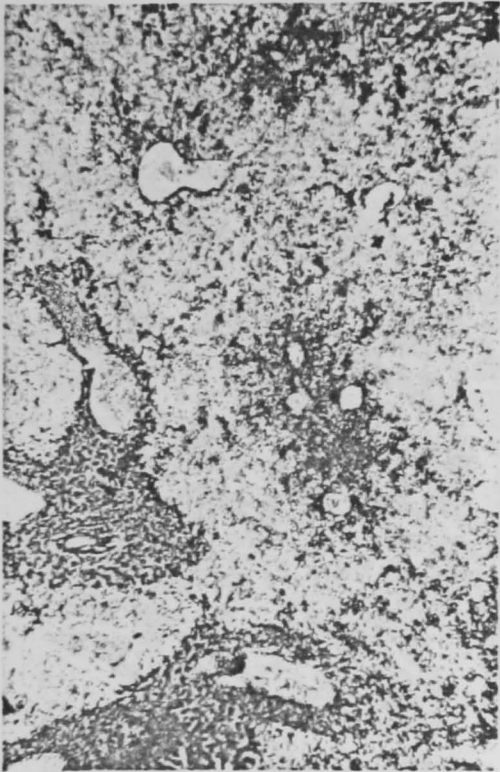


Figure 7:--Liver of trout force fed 5 mg/kg body weight of crude aflatoxin showing extensive liver cell necrosis with hemorrhage (light and stippled areas) and functional liver cells as islands of darker material. X60.



Figure 9:--Trout retina showing retinal elements isolated extensively due to severe edema. X150.

Dimethylsulfoxide pathology

Histological and hematological studies were completed on coho salmon fingerlings treated with dimethylsulfoxide (DMSO) by immersion, injection, and feeding. Significant changes observed in fish immersed in 16, 8, and 4 percent DMSO consisted mainly of hypoplasia of hemapoietic (head kidney) tissue (figs. 10 and 11); hyperplasia, clubbed gill lamellae and some necrosis of lamellar epithelium (fig. 12); also, some lamellar edema and exudation of plasma between gill lamellae (fig. 13). Edema and capillary congestion of meninges were also noted (fig. 14). Hematological changes found in fish immersed in 16, 12, and 8 percent DMSO consisted of a substantial increase in hematocrits and in numbers of both immature red and white blood cells. Degeneration was noted in some immature as well as in occasional mature erythrocytes. Leukocytosis was also common. No hematological changes were observed when DMSO was incorporated into the diet and fed at 0.5, 2.9, and 11.7 percent for a 6-week period.

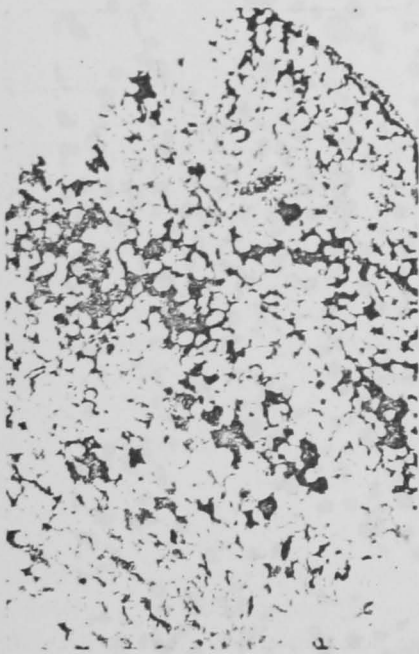


Figure 8:--Visceral fat with capillaries heavily congested from trout treated same as in figure 1. X100.

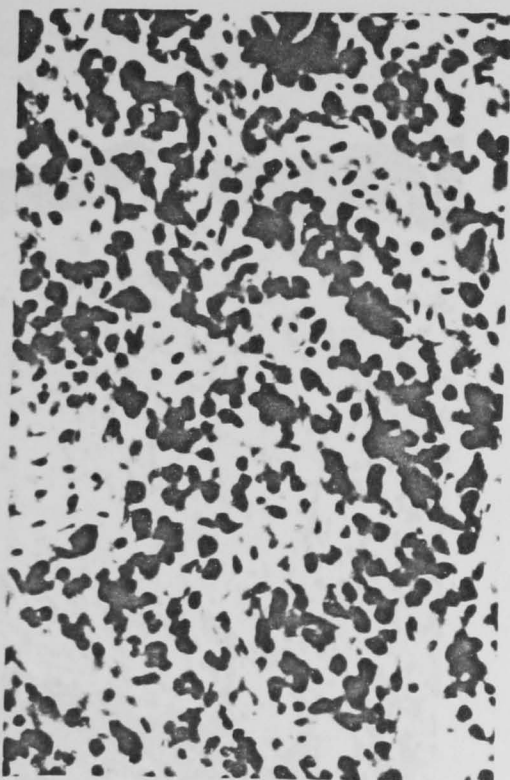


Figure 10:--Normal disposition of hemopoietic elements in hemopoietic (head) kidney of coho salmon. X400.

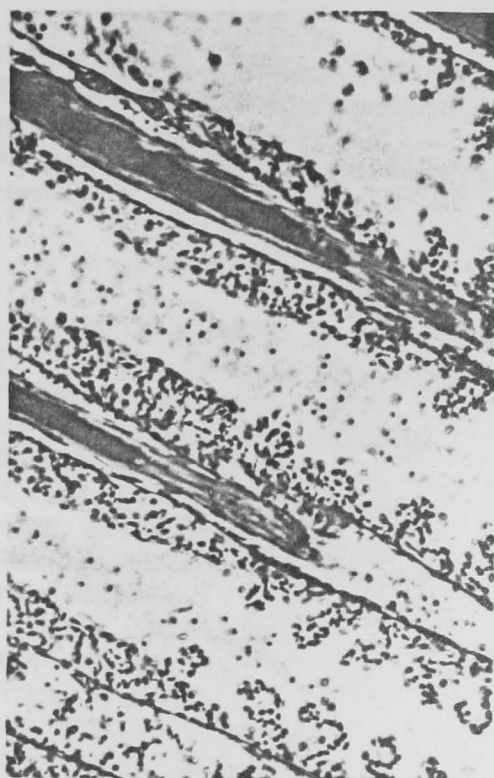


Figure 12:--Portions of three DMSO treated coho salmon gill filaments showing epithelial proliferation and sloughing of degenerate cells, and swollen clubbed gill lamellae. X200.

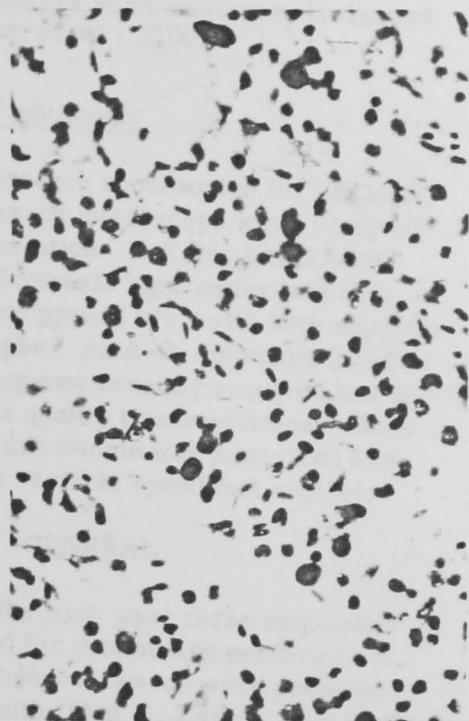


Figure 11:--Dispersed hemopoietic elements due to hypoplasia and edema in coho salmon treated with DMSO. X400.



Figure 13:--DMSO-treated coho salmon gills showing contracted lamellar capillaries and edema-distended lamellar epithelium. X100.

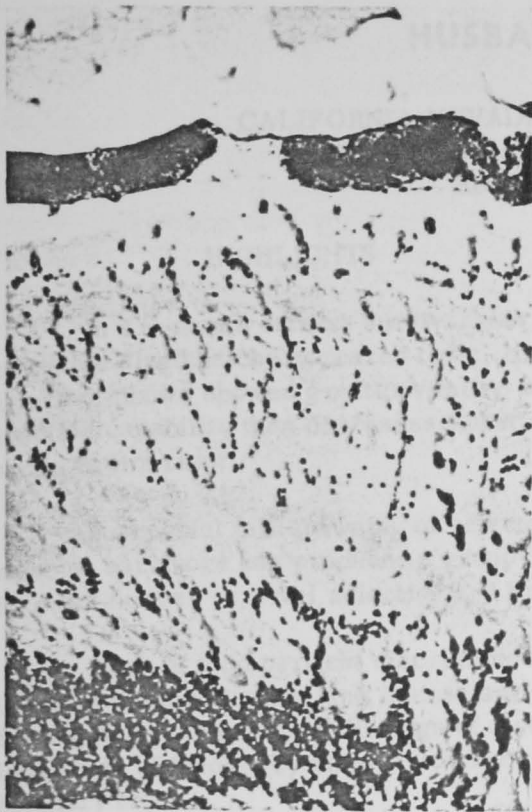


Figure 14:--DMSO-treated coho salmon brain and meninges showing extreme congestion of meningeal vessels with meninges distended from brain by edema. X100.

Acquisition of equipment

Fluorescence accessories for use with a Zeiss research microscope were received and put into operation. A Zeiss attachment camera with electronic light meter was also obtained and has proved it's worth with superior photomicrographs. An A-O-Automatic microscope knife sharpener recently acquired has resulted in higher quality tissue sections. Our O precision microtome was motorized and provided with a variable speed foot control.

GENERAL

During the past year three employees were recognized for outstanding performance. Superior Performance Awards were presented to Robert R. Smith, Max E. Larson, and Montie Peterson for consistent excellence of performance in the early hepatoma induction experiments conducted at Hagerman.

Mr. Pete Benville recently received an Incentive Award for modification of an existing piece of equipment to function in a more complicated system thus saving the cost of an expensive new instrument.

STAFF

- Dr. John E. Halver, Research Chemist
- Dr. Gilles J. La Roche, Research Chemist (WAE)
- Dr. Bradford Croston, Research Chemist
- Dr. Laurence M. Ashley, Fishery Biologist
- Warren E. Shanks, Research Chemist
- George D. Huestis, Administrative Assistant
- Robert R. Smith, Animal Husbandman
- George D. Gahimer, Fishery Research Tech.
- Pete E. Benville, Chemist
- Clarence L. Johnson, Physiologist
- Martha J. Tripp, Chemist
- Charlie E. Smith, Biologist
- Max L. Larson, Fishery Technician
- Myrna Morones, Library Assistant
- Dana N. Eshleman, Histopathology Technician
- Virginia L. Huestis, Scientific Secretary
- Montie C. Peterson, Fishery Aid
- Hazel J. Jones, Physical Science Technician
- Albert E. Merritt, Fishery Technician
- Carlle M. Southard, Fishery Aid
- Dale M. Odle, Clerk-Typist
- Margie M. Hoover, Fishery Aid
- Bill P. Carter, Fishery Aid
- Mary E. Cairns, Clerk-Typist
- Bonnie F. Ternahan, Fishery Aid
- Gordon C. Baker, Maintenance man
- Walter Brost, Maintenance man Foreman
- Albert W. Bell, Laborer
- Alton R. Anderson, Chemist
- Berle P. Bezzio, Student Trainee
- David L. Smith, Student Trainee

Although both groups began with indications of excellent vitality, the brown trout showed a large drop in hematological indices in November and a continuing condition loss associated with resulting poor overwinter survival. Rainbow trout which maintained better body picture and body condition through the winter survived at a considerably higher percentage. Spring feeding of both groups was moderate and nearly equal. Their average post-winter body conditions are illustrated in figure 1.

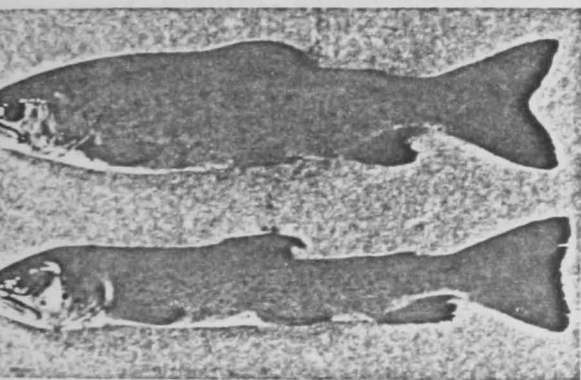


Figure 1:--Rainbow trout (upper) and Massachusetts brown trout (lower) after 9 months in the stream.

In management studies comparing them hatchery rainbow trout, hatchery reared Massachusetts brown trout have demonstrated potential for longer survival and extended contribution to angling in "fish-out" lakes. Further stream survival tests of these brown trout are planned.

SELECTION OF RAINBOW BROOD TROUT

A long-term program of breeding and survival selection with a single line of fall spawning rainbow trout was initiated in May. The general procedure is to select breeders from survivors of rigorous stream tests, continuing through several generations. Survival capabilities will be compared in yearly experiments and hemagglutination reactions will be studied to study divergence of the experimental parent stock types.

This year, 78 of 157 stream test survivors were graded out as superior and returned to Hot Creek State Trout Hatchery

for development to breeding condition. The return from wild to hatchery environment was made without difficulty except for a temporary reluctance to feed, but growth did permit spawning in October on the normal schedule. Eggs were slightly over one-half the size and about two-thirds the number expected from a hatchery reared female; however, the rates of hatching and subsequent growth were normal for the hatchery and fish for further stream testing and selection from this first filial generation is assured. The fish are now in the 75-100/ounce range and should reach planting size of 5/pound by May 1965. Control groups from the hatchery's production lots will be tested simultaneously with experimental groups to determine the extent of any survival improvement resulting from selection.

The foregoing projects were conducted in cooperation with the California Department of Fish and Game.

FEEDING HABITS OF STREAM STOCKED, HATCHERY REARED RAINBOW TROUT

Analyses of seasonal variations in food types and feeding intensity among groups of catchable sized trout from four California production hatcheries (Hot Creek, Moccasin Creek, Darrah Springs, and Moorehouse Springs) utilizing biweekly or monthly samples of stomach contents during the period May 1962 through May 1963, were completed in September.

Trout food in Convict Creek is supplied almost entirely by a limited number of species representing five orders of insects. In the summer period (May-October) 73 to 79 percent of all food was Diptera, Trichoptera, and terrestrial insects. In winter (November-May) 84 to 91 percent of total food intake was immature stages of Trichoptera, Ephemeroptera, and Diptera. Volume of food per stomach (monthly averages) varied from 0.12 to 0.90 cubic centimeters with heaviest feeding indicated for the months March-June. The high level of non-food materials (sand, leaves, twigs, etc.) in stomachs, an average of 40.6 percent for the year, showed non-discriminatory feeding to be customary.

The analyses did not indicate significant differences in feeding among trout groups from different hatcheries or stocks.

Lacustrine

comparison of contour nearly irregular and ill relationships with parison periphery were all Further unit of leaf that of this plankton as a com mental

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ALPINE WATERS INVESTIGATIONS

Stream seston studies, Laurel Creek

Lacustrine primary production

Shoal area productivity factors were compared for two adjacent high altitude lakes of contrasting morphometry: Edith, deep and nearly circular; Cloverleaf, shallow and irregular. Periphyton production, C^{14} fixation, and illumination levels showed no clear relationships within either lake. Between-lakes comparisons, however, indicated that rates of periphyton growth and C^{14} fixation in Cloverleaf were about five times greater than in Edith. Furthermore, the amount of carbon fixed per unit of seston was three times higher in Cloverleaf than in Edith. The most likely explanation of this phenomenon is that Cloverleaf phytoplankton displayed a more active metabolism as a consequence of more favorable environmental factors.

Laurel, an unusually productive lake for its location in terms of phytoplankton standing crop, receives water from two distinct geochemical sources. One inlet branch drains a metasedimentary rock and has a specific conductance of about 120 micromhos; the other branch arises in a granitic area and has a specific conductance of 50 micromhos. Cultures of native phytoplankton were established to determine the independent effects of these waters on lake metabolism. They consisted of quart mason jars filled with inlet water, inoculated with one milliliter of raw lake water, sealed with polyethylene film, and incubated in both lake shoal and stream environments. Analysis of growth in the cultures yielded the following information:

Place incubated	Growth period	Plankton Development, g-cal/l	
		Granitic inlet	Metasedimentary inlet
Lake Shoal	3 weeks	4.4	1.1
Lake Shoal	6 weeks	13.8	19.5
Creek	6 weeks	5.9	6.9

Granite derived water seems to have favored a greater initial algal growth, but larger quantities developed in the metasedimentary water at the end of 6 weeks. Superior growth in shoal cultures probably resulted from a higher level of illumination in that location.

Water samples from 8 stations along a 4-mile section of Laurel Creek were analyzed to determine seasonal and downstream changes in the concentration and composition of particulate organic matter. These data are summarized in figure 2; the area of each circle is proportional to the total quantity of seston per liter (extremes are station A, 0.3 mg/l and station B, 2.6 mg/l in August) and the segments of each circle show the relative amounts of major components comprising the seston total. Stations A and B represent the inlet and outlet of Laurel Lake, respectively. Other stations occur at intervals along the stream as it plunges more than 2,000 feet down a glaciated valley and terminal moraine. Sampling dates correspond to: high runoff, soon after the lake became ice-free (July); reduced flow and warm, late-summer conditions (August); low flow and cooling prior to onset of winter conditions (October).

Fluctuations in seston quantity were attributed to effluent lake plankton, ingestion by stream invertebrates, mechanical disruption of living cells, dilution with ground water (between stations D and E) and gradual accumulation of allochthonous debris in a downstream direction. Large numbers of filter-feeding simuliid larvae were present only at the August sampling. The indiscriminate feeding of these larvae was demonstrated by the analysis of their digestive tracts (isolated circles, figure 2) whose contents closely paralleled the composition of seston at the same locality.

Relative changes in numbers of diatom cells of the genera *Stephanodiscus* and *Asterionella* at each station were used as independent indices of algal cell losses by invertebrate ingestion and mechanical disruption, respectively.

In July the interstation decrease of *Stephanodiscus* varied only 2 to 7 percent; at the peak of simuliid development in August, a 60 percent decrease occurred between stations B and C (area of densest

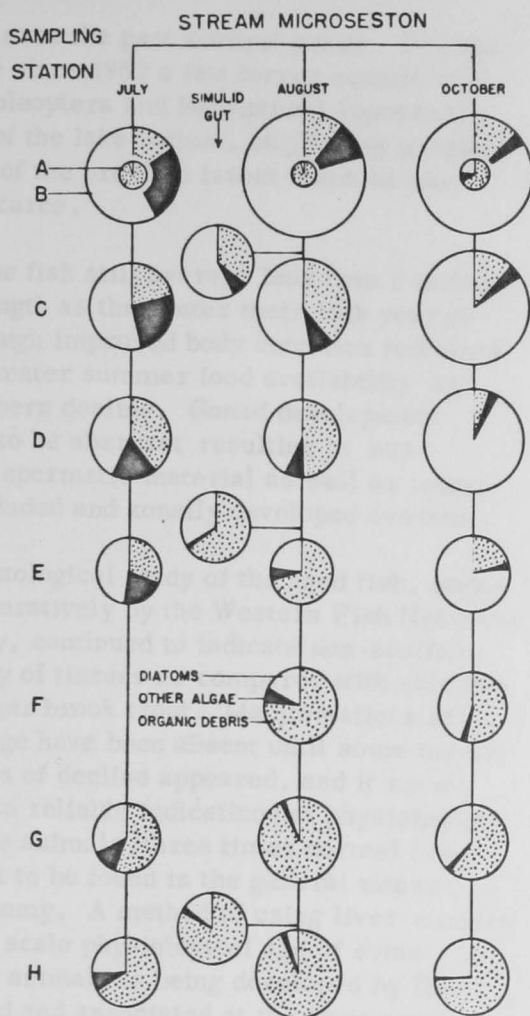


Figure 2:--Relative abundance and general composition of seston at eight stations along a 4-mile section of Laurel Creek in summer and autumn.

larval population). Relatively fragile *Asterionella* cells were abundant only in July samples. Interstation losses of these cells were large only where stream gradients exceeded 900 feet per mile (15 percent loss between G and H; 43 percent loss between C and D).

Environmental manipulation

Transplants of amphipods and crayfish into selected mountain lakes were delayed pending authorization of such introductions by the California Fish and Game Commission. Permission to proceed beyond the present limited observations was granted in October,

too late for consideration of an alpine program in 1964.

Experimental culture of the crayfish *Astacus klamathensis* was continued through the early part of the year for the purpose of studying cold-hardiness in this potential trout-food animal. Survival in troughs through the cold water season (October 1963 to June 1964) was 44 percent at a near-starvation level of feeding. Water temperatures averaged in the high 30's and ranged down to 32° F.

Major activity in this project was the examination of waters known to have been stocked with amphipods (*Gammarus* and *Hyaella*) in efforts to improve trout food conditions. So far, 32 lakes have been investigated by combinations of visual examination, dredging, water analysis and fish stomach analysis; of these, 14 contain self-sustaining populations of amphipods that contribute as much as 78 percent of food organisms found in trout stomachs. Reasons for success in some waters and failure in others are less clear than could be desired, although factors of shelter and predation intensity appear to be most important with present trout populations. Water chemistry is important as a determinant of the plant shelter and food substrate attainable, and a few of the lakes surveyed probably reach lower limits for the support of amphipods in more than slight density. Chemically lethal situations, except for acid bogs and the low-oxygen bottom areas of deep water, are rarely found in alpine lake basins. A large consideration in the evaluation of stocking failures of earlier years (1920-1940) must remain unknown that is, definite information as to persistence of the stocked species for even short periods after transplant is lacking and cannot now be obtained. There seems a good possibility, based on this year's lake survey observations, that many sites for introduction were chosen without sufficient regard for shelter.

BIOLOGY OF STUNTED, 14-YEAR-OLD BROOK TROUT

A September inspection of the small, 11,000-foot-elevation cirque lake containing the few remaining trout of this known-age population revealed slight change from conditions

observed over the past several years. For the first time since 1952 a few larger aquatic food forms (Coleoptera and Hemiptera) appeared in samples of the lake bottom, suggesting possible recovery of the pre-fish insect fauna as trout become scarce.

The fish still average less than 8 inches in total length as they enter their 15th year of life, although improved body condition indicates slightly greater summer food availability as trout numbers decline. Gonad development continues to be aberrant resulting in non-functional spermatic material as well as membrane-occluded and zonally developed ovaries.

Histological study of the aged fish, undertaken cooperatively by the Western Fish Nutrition Laboratory, continued to indicate non-senility of a variety of tissues as compared with controls from younger brook trout. Manifestations of advanced age have been absent until some terminal features of decline appeared, and it seems that a key to reliable indications of physiological age in these animals (three times normal life-span) is not to be found in the general microscopic anatomy. A method of using liver-enzyme strength to scale physiological age of some short-lived animals is being developed by Dr. Roy Walford and associates at the Department of Pathology, U.C.L.A. School of Medicine. Possibilities for collaborative study of aging in trout have been discussed.

This year's attempts to induce rapid aging by increasing metabolism in specimens transferred to the laboratory had no clearly definable effects other than the previously observed resumption of normal growth with feeding. A few fish developed cataract or other loss of photoreceptivity with resulting loss of skin pigment control. Lethargy, coma, and death gradually followed but no histological evidence of general breakdown was found.

Although natural reproduction has not been possible in Bunny Lake, fertilization of part of the eggs from a 14-year female reared at the laboratory since October 1963 was accomplished in November of this year. Semen from a similarly laboratory nurtured male effected fertilization of eggs from hatchery brook

trout in a second trial. In these reciprocal matings of old Bunny Lake fish with younger hatchery broodstock deficiencies in germinal cells resulted in low rates of fertilization and hatching and unusually high incidence of deformed larvae. In a third trial, some eggs of the 14-year female were fertilized (at about 40 percent) by sperm from an aged male; of the fertilized ova, all but two died in early embryonic stages.

A report on these record-age trout is in preparation.

LIMNOLOGICAL METHODS

Coprecipitation of dissolved organic matter

The precipitation of dissolved organic matter with ferric and aluminum hydroxides was investigated as an adjunct technique in the oxidative determination of organic matter in natural waters. It was hoped that this process would obviate two difficulties of the analysis, concentration of organic material and removal of interfering chlorides. Preliminary tests showed that neither precipitating agent interfered with the oxidative reaction, but since the ferric hydroxide gel was easier to handle, it was employed in subsequent studies.

Among the variables investigated which affect the efficiency of coprecipitation, pH had the greatest influence. Representative curves for the recovery of various organic compounds at different pH values are given in figure 3. The response of stearate was similar to gelatin and curves for sucrose and glycine were like that of glucose. In general, optimum pH was near neutrality and recoveries were greater for compounds of higher molecular weight. The effectiveness of coprecipitation on natural waters was examined next, using a Lake Crowley sample and comparing the results to an "absolute" standard obtained from freeze dried residues of the same sample. The results (fig. 3) indicated a maximum recovery of dissolved organic matter near 50 percent between pH 6 and 7.

Final tests were conducted to determine the effect of coprecipitation on chloride removal using marine waters. Despite repeated rinsings, residual chloride in the precipitate remained at

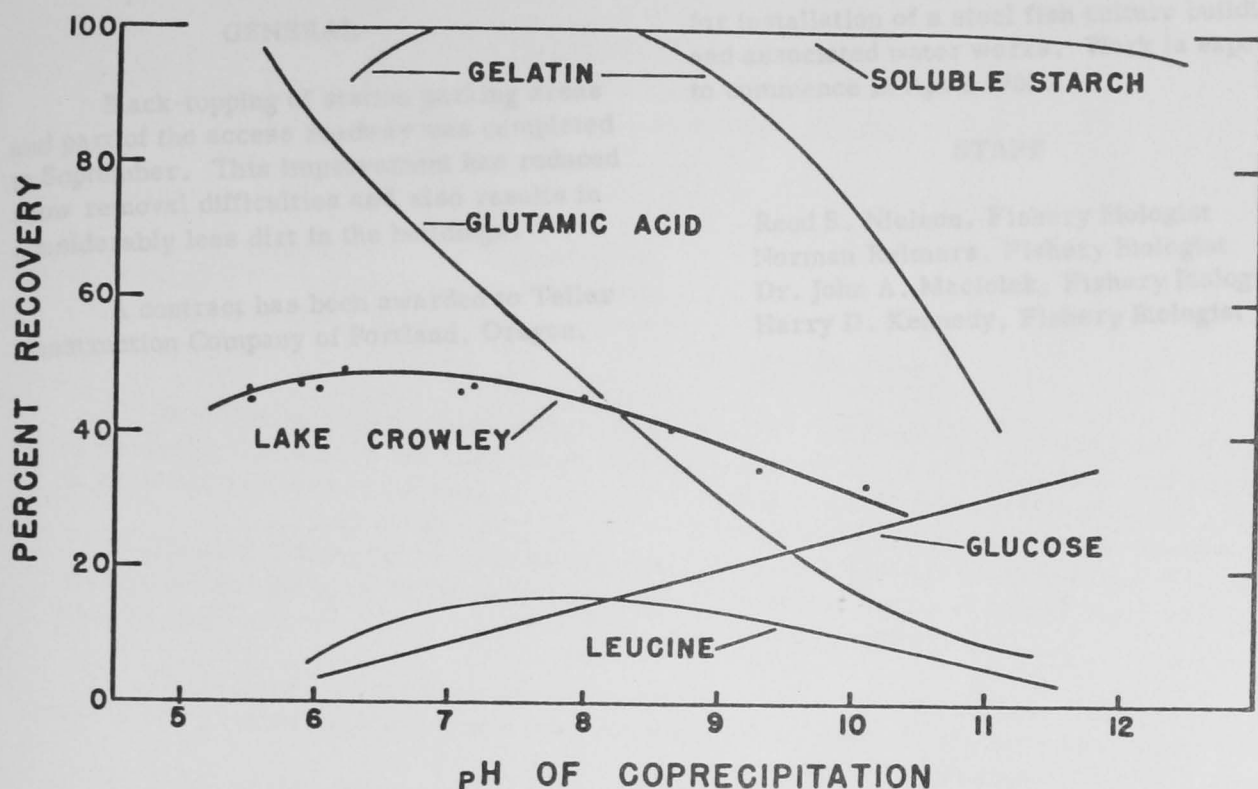


Figure 3:--Coprecipitation recovery of some organic compounds in solution and of dissolved organic matter in lake water at different pH values.

interfering levels. Analysis was then adjusted to measure dissolved organic carbon in the precipitate at sub-milligram levels via non-quantitative oxidation. This technique was applied to offshore euphotic waters on a Scripps Institution cruise to the area around southern Baja California. Nine stations were established in two types of waters; three in warm, clear, unproductive tropical water and six in a cool turbid upwelling. Six strata having depths from 5 to 47 meters in clear water and 2 to 12.5 meters in turbid water were sampled at each station. Results of the coprecipitated carbon analyses, averaged with respect to station and water type, are compared to coincident measurements of seston and chlorophyll below:

Coprecipitated carbon showed less difference between water types than either seston or chlorophyll. Although these values for dissolved organic carbon are within the generally accepted range for marine waters, they are believed to be excessive because of previously mentioned recovery efficiencies of ferric hydroxide. This may be the result of inorganic carbon interference. The coprecipitation technique therefore appears unsatisfactory for quantitative analyses of dissolved organic matter in either fresh or saline waters.

Water type	Per liter values		
	Dissolved organic carbon, milligrams	Total seston Milligrams	Chlorophyll fluorescence units
Clear tropical	3.20	0.28	0.17
Turbid upwelling	4.31	1.07	9.12

GENERAL

Black-topping of station parking areas and part of the access roadway was completed in September. This improvement has reduced snow removal difficulties and also results in considerably less dirt in the buildings.

A contract has been awarded to Teller Construction Company of Portland, Oregon,

for installation of a steel fish culture building and associated water works. Work is expected to commence in April 1965.

STAFF

Reed S. Nielson, Fishery Biologist
Norman Reimers, Fishery Biologist
Dr. John A. Maciolek, Fishery Biologist
Harry D. Kennedy, Fishery Biologist

FISH FARMING EXPERIMENTAL STATION

Stuttgart, Arkansas

James H. Stevenson, Director

HIGHLIGHTS

Data collected during a 2-year period show that the black X bigmouth buffalo hybrid grew faster than other buffalo hybrids or their parent species.

Modification of the trough-culture method of producing flathead catfish fingerlings resulted in a marked increase in survival.

Channel X blue catfish hybrids outgrew the channel and the blue catfishes by 15 and 65 percent, respectively.

Grass carp held at the station grew from a size of 4 grams to 1,816 grams during the year.

Dylox proved effective for the control of all stages of *Lernaea* when applied to ponds weekly at the rate of 0.25 ppm active ingredient for one month.

Dry feed pellets are more water stable if ingredients are ground after mixing and if organic flours are added as binders.

Zooplankton populations were significantly increased by adding nitrogen fertilizer in well water but not in surface water.

Karmex effectively controlled filamentous algae when applied at the rate of 0.75 pounds per surface acre.

The herbicide Casoron controlled all rooted aquatic vegetation in pond tests at rates above 0.5 ppm.

The amount of organic carbon and inorganic phosphorus found in the bottom soil of ponds is influenced by the source of water and the amount of nitrogenous fertilizer applied.

There is a buildup of inorganic carbon in the soil of ponds receiving high rates of nitrogenous fertilizers.

The organic phosphorus fraction of the soil is greater in ponds filled with surface water.

FISH CULTURE

Hybridization

Second-year growth rates of black X bigmouth, black X smallmouth, and smallmouth X bigmouth buffalo hybrids show that all three crosses outgrew their parent species. The black X bigmouth hybrid demonstrated the greatest degree of hybrid vigor by outgrowing the other hybrids.

To measure their relative growth rates, equal numbers of each cross and of their parent species were given identifying fin clips and stocked together in duplicate 1.0-acre ponds. At the end of the second season, growth data were subjected to analysis of variance. The black X bigmouth hybrid was consistently 33 percent heavier and 10 percent longer than the parent species. A summation of these data was presented at the Eighteenth Annual Conference of Southeastern Game and Fish Commissioners.

During their second year the channel X blue catfish hybrids were stocked with equal numbers of their parent species in each of seven ponds. After a growing season of 225 days, growth rates of the three groups were calculated and compared. The hybrid outgrew the channel catfish and blue catfish by 15 and 65 percent, respectively.

Morphological examination showed the hybrid to be intermediate to the channel and blue catfishes in body and head conformation, and anal fin ray count. The hybrids were spotted similarly to the channel catfish but closely approached the blue catfish in skin coloration. Another character noted was that the two medial ventral barbels were completely white.

Flathead catfish

Further studies on the rearing of flathead catfish fingerlings in aluminum troughs resulted in the modification of the cultural procedures described last year. Subsequently, the survival rate was increased from 60 to 95 percent and the growth rate improved to 2.5 inches in 45 days compared to the previous rate of 2 inches in 80 days.

A diet of 40 percent beef liver, 40 percent fish flesh, and 20 percent bread crumbs by volume, was used. The ingredients were ground while partially frozen and bread crumbs were added to absorb blood and juices. No other foods were used during the study. A temperature of 75° F. was maintained.

Preliminary studies were initiated to evaluate the predaceousness of the flathead catfish. A 0.25-acre pond was divided with polyethylene barrier through the center from the shallow to the deep end. Equal numbers of fathead and golden shiner minnows, green sunfish, bluegills, orangespotted sunfish, Tilapia, buffaloes, and crappies were stocked on either side of the partition. An addition of six 2-year-old (2-pound) flathead catfish was made to one side. By fall the population of other fish on the side containing the catfish was reduced by 53 percent. The number of crappies was greatly reduced but their average size and total weight increased. The average gain by the catfish was 2.2 pounds.

In another experiment, 2-year-old flathead catfish were stocked at the rate of 46 per acre and given 15 pounds of fathead minnows for forage. The average gain of the catfish was 0.5 pounds.

Grass carp

Early growth of the grass carp, Ctenopharyngodon idellus, compared favorably with that reported in semi-tropical countries. During their first year at the station, the fish grew from a length of 8 centimeters and a weight of 4 grams to a length of 50 centimeters and a weight of 1,816 grams. If the present rate of growth continues, it seems likely that some individuals will reach sexual maturity in 1966.

Observations on their feeding habits suggest that they are not strictly herbivorous if other food is available. Fish stocked at the rate of 24 per acre failed to eliminate aquatic plants when supplemental food was provided.

Freshwater shrimp

Preliminary studies on the culture of the freshwater shrimp, Macrobrachium ohione, were completed during the year. Adult shrimp were successfully held in ponds and aquaria. Spawns in aquaria failed to survive despite attempts to attain a satisfactory environment. Spawns in ponds were achieved and larval shrimp appear to be growing well. Data on growth, development, and molting have been collected and are being summarized.

Crayfish

Studies on the culture of two crayfishes, Procambarus clarkii and P. blandingii in flooded rice fields are underway. Test plots have been stocked to study the growth of these species in this locality. Spawning has been observed and growth rates are being followed.

PARASITES AND DISEASES

Drug clearance

Correspondence with representatives of the Food and Drug Administration produced a clarification of regulations concerning the inclusion of medicants in commercial fish feeds. A recent opinion stated that the FDA is not objecting to the use of drugs in feeds limited to non-food species. No drugs are cleared for use in feeds provided to fishes intended for human consumption.

Lernaea control

During the 1964 calendar year, fourteen 0.1-acre ponds were used in experiments relating to the control of the anchor parasite, Lernaea cyprinacea. Eight ponds were devoted to replicated studies in which selected organophosphate insecticides were included in the daily ration and two ponds were left untreated to serve as controls.

It was hoped that this technique would effectively control the parasite by systemic action similar to current controls for cattle grubs. Preliminary work in 1963 had indicated favorable results although control did not reach desired levels at that time.

During 1964, increased levels of the insecticides were fed in the daily rations. If control was not achieved after 4 weeks, the dosage was increased. All ponds were stocked at the rate of 40 pounds of golden shiner broodfish per acre on April 1 and supplemental feed was provided daily, Monday through Friday. Barnyard manure was added initially to the ponds to encourage plankton growth and commercial fertilizers were added to maintain secchi disc readings of less than 18 inches. Weekly observations were made to insure survival and spawning success.

Dylox, Menazon, and Co-Ral were fed at rates up to 4,000 ppm; levels up to 8,000 ppm of Ronnel were tested. Results obtained in these trials, summarized in table 1, indicate that only Dylox merits further consideration as a systemic control for *Lernaea*. Although successful spawning occurred in most ponds, the number of fish produced was not good. Reasons for this low production are unknown.

Four 0.1-acre ponds were used to test the relative merits of adding chemicals to the pond water as a control for the anchor parasite. These ponds were stocked as in the above tests but with fish from another source. Management of the ponds was similar.

Dylox, methyl parathion, and benzene hexachloride as water treatments were compared. Since the standard treatment for anchor parasites has been to apply 0.5 ppm of 12 percent active wettable powder benzene hexachloride (BHC) at 5-day intervals for one month, treatments were made for a similar period with each compound. Dylox (50 percent wettable powder) was applied at the rate of 0.25 ppm (active) and methyl parathion as a 2 pounds per gallon formulation was applied at the rate of 0.125 ppm (active) per application. Results of the applications are provided in table 2. All compounds gave good control but only in the Dylox-treated

pond was eradication achieved. In the BHC-treated pond a 5 percent incidence was recorded at the end of the treatment period. Increasing the level to 1.0 ppm eliminated the parasites. The parasite level in the methyl parathion treated pond was 8 percent and continued treatment for another month did not eliminate the parasites from the pond.

In 1963, only two treatments with Dylox were needed to eliminate the parasites. Four treatments were required in 1964. A check of the various possible factors which might have affected the results revealed only one major difference between the two years. In 1964, maximum water temperatures during the test interval reached 96°F. at a depth of 2 feet as compared to only 85°F. in 1963.

Since organophosphates are affected by elevated temperatures and alkaline conditions, it is possible that the reduced effectiveness of both Dylox and methyl parathion was due to a rapid breakdown of the pesticides. The range of pH values of the pond water during the test period was 8.1 to 9.5.

Spawning occurred in all ponds and growth of the fry was excellent. Although production figures did not reach desired levels, harvest data for the test ponds were fairly uniform.

All chemicals affected the biota in the ponds. Benzene hexachloride eliminated the zooplankton. Methyl parathion and Dylox reduced the incidence of zooplankton but it was again present in great abundance 2 weeks after the last treatments. Chironomid larvae were abundant in the bottom mud of all ponds throughout the test interval. Aquatic insects were eliminated in the pond treated with benzene hexachloride when the treatment level was raised to 1.0 ppm.

Ichthyophthirius control

Ichthyophthiriasis appeared in experimenta stock at the station on October 30 and studies on this disease were resumed. Daily treatments with 200 ppm formalin for one hour gave complete control of the disease in tank situations. Replacing the formalin applications on alternate days

Table 1:--Results of feeding four insecticides in the daily ration of golden shiners for 16 weeks.

Figures A and B represent 2 ponds for each compound.

PARAMETER	CO-RAL	DYLOX	MENAZON	RONNEL	UNTREATED CONTROL
	4000 ppm	4000 ppm	4000 ppm	8000 ppm	-----
Lbs. stocked per acre	40	40	40	40	40
Size stocked	20/lb	20/lb	20/lb	20/lb	20/lb
Fry survival	A) None B) None	A) Fair B) Good	A) Fair B) Fair	A) None B) Excellent	A) Fair B) Fair
Lbs. per acre harvested	A) None B) None	195 295	286 199	58 585	204 252
Size at harvest	A) ---- B) ----	A) 21/lb B) 21/lb	A) 19/lb B) 21/lb	A) 25/lb B) 104/lb	A) 18/lb B) 22/lb
Parasite control	Poor	Excellent	Poor	Poor	-----
Effects on:					
Copepods	Not affected	Not affected	Not affected	Not affected	-----
Cladocera	Reduced	Eliminated	Not affected	Eliminated	-----
Rotifers	Not affected	Not affected	Not affected	Not affected	-----
Bottom organisms	Not affected	Not affected	Not affected	Not affected	-----

Table 2:--Effects of applications of three insecticides in ponds stocked with golden shiners.

Parameter	Dylox	BHC	Methyl Parathion	Untreated
Treatment level (ppm)	0.25	0.5 ^{1/}	0.125	-----
Treatment interval (days)	7	5	5	-----
Lb/acre Stocked	40	40	40	40
Lb/acre Harvested	332	282	325	308
Fry Survival ^{2/}	Good	Fair	Good	Fair
Zooplankton	Reduced ^{3/}	Eliminated	Reduced ^{3/}	Abundant
Bottom Organisms	Abundant	Abundant	Abundant	Abundant
Parasite Control	Excellent	Good	Good	-----

^{1/} Treated with 0.5 ppm of a 12% active wettable powder.

^{2/} Based on increased number of fish harvest.

^{3/} Zooplankton was again evident in great abundance two weeks after the last application.

with treatments of 0.3 ppm malachite green for one hour also gave complete control. No recurrences were noted with either technique.

Tests using Sulquin (sodium sulfaquinoxaline) and Flagyl (metronidazole) as water additives were inconclusive. In certain tests, Sulquin suppressed the disease but the results could not be consistently reproduced. Catfish with visible symptoms of Ichthyophthiriasis refused to eat medicated feeds.

Diagnostic services

During 1964, 165 requests for diagnostic services were received. There was a direct relationship between seasonal outbreaks of disease and the number of requests. Although most of the fish examined came from Arkansas, requests were received from 11 States in an area bordered on the west by Montana, on the north by Wisconsin, the south by Texas and on the east by South Carolina.

NUTRITION

Feed processing

Meals for minnow fry, ground twice through a screen with openings 1/16-inch in diameter, contained a significant portion of material too large for immediate ingestion. However, field tests of four formulas, texturized by twice-grinding, produced excellent survival and growth. Laboratory tests indicated that minnow fry feeds should be sieved through a U.S. No. 80 sieve (177 microns) for 100 percent acceptance.

Pellet stability in water was increased by grinding test feeds and by the addition of organic flours. Several products were used successfully as binders including soy flour, dried wood pulp liquor, ground guar meal and rice mill dust. The latter product was obtained locally by grinding rice hulls and using the material sieved through a U.S. No. 80 screen. Several other products recommended as binders for dry pellets were tried as pellet stabilizers, but these hastened hydration and pellet disintegration.

Formulation

Station formula No. 11 was modified slightly from formula No. 10 (1963 Annual Report) to improve water stability and decrease cost. The average proximate analysis of this ration was as follows: crude protein - 33 percent, crude fat - 6 percent, crude fiber - 12 percent, ash - 9 percent, nitrogen-free extract - 30 percent, moisture - 10 percent. Pellet stability, as measured by the percentage retained on a screen when placed in water, was 90 percent using a feed mixture of purchased ingredients, and 93 percent using a mixture ground through a 1/16-inch screen. The replacement of rice hulls with rice mill dust resulted in further improvement in water stability.

A vitamin premix was added to ration No. 11 and the fortified diet fed to duplicate ponds of channel catfish fingerlings. Fish sampled for 60 days showed no difference in weight gains between those on the fortified and control rations. All subsequent samplings and final weights showed better growth by the fish on the fortified ration.

Four formulas were manufactured from essentially the same ingredients, each formula containing a high level of one nutrient class, either protein, fat, starch or fiber. These were fed to 3-year-old channel catfish daily at the rate of 2 percent body weight unless feeding activity was found to be sluggish. Table 3 shows growth data of fish on these feeds. A mid-season change in pellet size from 1/8-inch diameter and 7/8-inch length to 1/4-inch diameter and 1/2-inch length was readily accepted. Three-year-old channel catfish had excellent survival and attained a maximum weight of 2.8 pounds. Feed costs for 3-year-olds were twice those for younger fish. Last year these test fish were on a standard ration and gained an average of 1.1 pounds using 1.4 pounds of feed. They required slightly less than 1,400 kilocalories of feed per pound of weight gain and produced an average of 1,550 pounds gain per acre of pond.

The relative value of protein concentrates as measured by growth of channel catfish and goldfish fingerlings in aquaria at water temperatures from 66° to 68°F., were as follows:

Table 3:--Growth data (1964 season) for 3-year-old channel catfish stocked 1,500 per acre on rations high in a single nutrient class.

Type of ration	Final weight (lbs.)	Gain (lbs.)	Conversion	Gains/acre (lbs.)
High-Protein	2.5	1.40	3.1	2,030
High-Fat	2.6	1.46	3.2	2,070
High-Starch	2.8	1.58	3.3	2,250
High-Fiber	2.5	1.34	3.7	1,930

Table 4:--Weight gains and feed conversion of 2-year-old blue, white, and channel catfish during the 1964 growing season.

Species	Stocking rate	Av. Wt. grams	Feed Conversion	Condition factor
White	1,500	377	-	0.94
	2,500	413	3.4	0.94
Blue	1,500	324	2.7	0.65
	2,500	346	2.7	0.60
Channel (with vitamins)	1,500	528	2.2	0.55
	2,500	483	2.3	0.65
Channel (without vitamins)	1,500	355	2.8	0.66
	2,500	346	2.8	0.59

casein - 100 percent, fish meal - 120 percent, soybean meal - 49 percent, and feather meal - 43 percent. Current results indicate that soybean meal is much better utilized at temperatures of 80° to 84°F. than at lower temperatures.

Growth tests

Channel, blue, and white catfish fingerlings, stocked at 1,500 and 2,500 per acre, were fed a supplemental feed (Ration 11). Table 4 shows average weight, feed conversion, and condition factors at the end of the second year. Channel catfish on a complete feed were compared with those on a supplemental feed. Weight gains in descending order are: channel catfish

on complete feed, white catfish, channel catfish and blue catfish on supplemental feed.

Single 0.1-acre ponds were used to grow channel catfish fingerlings on rations supplemented with thyro-protein, proteolytic enzymes and diastatic enzymes. Fish receiving diastatic enzymes had the most weight increase.

Four-year channel catfish stocked at the rate of 1,000 per acre weighed 2.0 pounds at the beginning of the season. May samples secured fish which averaged 2.5 pounds. No additional gains were made in these fish during the season. Apparently the space factor stress kept the fish from gaining, although constant attention was

given to water quality and supplemental feed requirement.

Data on the growth of blue catfish are limited. A comparison was made between fingerlings being reared in 0.25-acre ponds at the station and fish stocked at the same rate (1,500/acre) in 2-acre ponds on a local fish farm. At the end of one growing season, the blue catfish measured 13.0 inches (0.78 lbs.) at the station compared to 13.3 inches and 0.84 pounds at the fish farm.

Biochemical analyses

Proximate analyses of fish carcass, fish feed and gastro-intestinal contents were made on 2-year-old channel catfish in ponds for the purposes of measuring (1) time for feed digestion and (2) digestibility of nutrient classes. This work is in progress. At water temperatures of 72-75°F., protein and fat were digested from ration No. 11 at 60 and 75 percent, nitrogen-free extract about 25 percent, ash and fiber seemed to be inert. Thirty minutes after feeding, the stomach contents of fish contained large amounts of pellets and water. After 12 hours the stomach was empty and 24 hours after feeding the entire gastro-intestinal tract was empty.

LIMNOLOGY

Nitrogen and phosphorus fertilization

Pond fertilization studies during 1963 indicated that nitrogen fertilization influenced zooplankton production to a larger extent than did phosphorus. The investigation was continued during this year using two rates of nitrogen fertilizer with a single rate of phosphorus. The test was expanded to include both well and surface water sources. Duplicate ponds from each water source were fertilized with each rate of nitrogen. The test fertilizer mixtures were 4 - 24 - 2 and 12 - 24 - 2. Nine applications of 100 pounds per surface acre were used from April through September. Samples were collected biweekly during the period for chemical analysis and zooplankton counts.

All of the test ponds were stocked alike and received 220 catfish, 50 buffaloes, 150 crappies, 20 paddlefish, and 20 pounds of forage fish per acre. Individuals of each species were weighed and measured prior to stocking and their growth was recorded during the November harvest.

Increasing the nitrogen fertilizer from 4 to 12 pounds per acre per application had no significant effect upon the concentration of ammonium nitrogen or ortho-phosphate in either the well or surface water ponds. Fish production was not different in either type of water or at either fertilizer rate.

Soil and water relations

Ground and surface water held over pond soils in aquaria showed changes in quality during 5 weeks of impoundment. The changes were influenced by constituents contained in the soils and by the initial mineral content of the water. Compared to ground water with high mineral content, surface water of low mineral content permitted greater quantities of ions to pass from the soils.

Soils influenced specific conductance, pH, alkalinity, calcium, magnesium, ammonium and nitrate nitrogen, and turbidity. Soil from an old fish pond contributed more ions, especially calcium and nitrate nitrogen, to ground and surface water than did soil from a newly constructed pond. Surface water decreased in turbidity and increased in alkalinity, specific conductance, and magnesium when impounded over soil from an old pond.

The nutrients existing in the old and new soil were determined by two methods: (1) "readily exchangeable" nutrients were determined by analyses of dilute acid and salt extractions; (2) "water soluble" nutrients were determined by analyses of saturated soil extracts. The differential responses of ground and surface waters impounded over old and new soil could not be accounted for by readily exchangeable nutrients because: (1) the calcium contents of the soils were similar, yet old soil contributed more calcium to both waters than new soil; (2) the magnesium content of new soil was greater, but

old soil contributed more magnesium ions to surface water. The saturated soil extract analyses indicated that old soil contained more calcium, magnesium, and nitrate nitrogen, and yielded higher values for alkalinity and specific conductance than the new soil. These same constituents were found in greater amounts in both waters over old soil. These analyses of saturated soil extracts indicate differential responses of water to soils.

Soil tests of station ponds

Soil samples have been collected semi-annually for the past 2 years from all station ponds. "Soil test values" of these samples were determined by analyses of dilute acid and salt extractions at the Soils Testing Laboratory, University of Arkansas, Fayetteville. The constituents determined included: pH, organic matter, specific conductance, phosphorus, calcium, magnesium, potassium, sodium, iron, and manganese.

The analyses of the 1964 samples have not been completed but the following observations are evident; since preimpoundment the pH, and the phosphorus, potassium, calcium, magnesium, iron, and manganese content of the pond soils have increased and the sodium content has decreased.

Underwater photometer

An underwater photometer has been designed and constructed to study the effects of light penetration on fish production. Preliminary testing indicates that it is highly sensitive and capable of furnishing much of the required information. Calibration of this instrument is currently underway and it is hoped that it will make possible the determination of absolute light intensity values at varying water depths.

Artificial oxygenation

Tests conducted on a holding tank aeration device were encouraging. Bottled oxygen supplied under a hood arrangement in the holding tank produced oxygen concentrations of 13 to 16 ppm at 60° to 63°F. Comparative tests with mechanical agitation yielded oxygen concentrations of 6 to 8

ppm at the same temperatures. Small holding operations and hauling units might benefit by this type aeration.

AGRONOMIC RESEARCH IN FISH-RICE ROTATIONS

In cooperation with the University of Arkansas, research on the relationships between fish farming and rice production was continued. A greenhouse study was conducted in 1964 on silt loam soils from 90 different fields which had been occupied by either rice, lespedeza, cotton, soybeans, or reservoir water (irrigation water and/or fish) during the previous year. The purpose of the test was to compare the effect of the past year's cropping history on (a) nitrogen soil test values by the incubation method and (b) the amount of available nitrogen present in soil samples. The amount of available nitrogen present in soil samples initially and after 6 days incubation in the laboratory decreased according to past cropping history in the order of reservoir samples > lespedeza field samples > rice field samples > soybean field samples > cotton field samples. The amount of available nitrogen contained in reservoir samples were nearly 4 times greater than amounts contained in cotton field samples. Statistical analyses to compare the effect of past cropping history on soil test values are underway.

Field tests were conducted at five fish farms having clay soils to obtain data for nitrogen soil test comparisons. Available nitrogen after 6 days incubation was more closely related to grain yields than were soil organic matter values. These results confirm those obtained previously on silt loam soils.

Total acreages used in fish farming appeared to have changed little, if any, since 1960 while the distribution of acreage used for culture of different species changed greatly. The acreage used for minnow and catfish production increased during the years 1960 through 1963 while the acreage used for the production of buffalo and combinations of buffalo with other species decreased.

POND MANAGEMENT

Species combinations and stocking rates

Several stocking rates of combined species of fish were used to determine the optimal growth of each species and the maximum yield of marketable size fish. Fingerlings were stocked in newly constructed 1.0-acre ponds in early summer of 1963 and harvested in the fall of 1964. The ponds were fertilized with commercial fertilizer to maintain a heavy plankton bloom. Data were collected of the water chemistry, plankton populations, and soil composition and compared for changes in pond properties.

The highest yield (709 pounds) and best individual growth were obtained from a 1.0-acre pond stocked at the following rates: 125 bigmouth buffaloes, 50 channel catfish, 50 white catfish, 100 crappies, 25 flathead catfish, and 5 Israeli carp. No supplemental feed was provided. Although the average weight of the buffaloes was 4.5 pounds, a large percentage were too small to bring a premium market price. Channel catfish averaged 1.4 pounds in weight and white catfish averaged 0.9 pounds.

Lower stocking rates of buffaloes in other ponds failed to produce larger fish. Higher stocking rates of the same six species in a 1.0-acre pond provided with supplemental feed showed a total yield of 3,000 pounds. The buffaloes stocked at a rate of 350 per acre had an average weight of 4.0 pounds. The other species were all of marketable size and showed growth similar to that expected in a supplemental feeding program. An intensive type of management, using channel catfish alone and with supplemental feeding showed the best feed conversion and proved to be the most profitable. A 1.0-acre pond, stocked with 1,500 channel catfish produced 3,600 pounds of 2.5-pound fish after 18 months.

The value of the buffalo in a combined culture program cannot be ignored. Use of hybrids or selected individuals may provide a better growing fish.

Chemical weed control

In 1963 the herbicide Casoron gave promise of being effective against most rooted aquatic plants encountered in station ponds. All preliminary tests were made by applying the granular herbicide to the soil before filling. Testing this year was expanded to include water applications along with soil treatments. Eleven ponds received either water or soil treatment at rates from 0.5 to 3.0 ppm Casoron. Ponds that were water treated had established growths of one or more of the following plants: *Najas*, *Chara*, *Heteranthera*, *Sagittaria*, *Jussiaea*. Ponds that were soil treated had a previous history of supporting the same plants. In all ponds good control of all rooted plants was obtained except at the 0.5 ppm rate. Soil and water treatments were equally effective. The existing plants at time of treatment were killed but re-growth of *Najas* and *Jussiaea* was evident after one month. Other treated ponds contained no rooted plants during the entire year. Although good control of rooted aquatics was obtained, no control of filamentous algae was exhibited by Casoron.

Excessive blooms of blue-green algae have been experienced in ponds used for catfish feeding tests. Preliminary tests in 1962 and 1963 showed that Simazine was effective in retarding such blooms. Six ponds being used for feeding tests were selected for duplicate treatments of 0.3, 0.5, and 1.0 ppm Simazine. Good to excellent bloom control was obtained in all ponds during the entire year. At the 0.3 and 0.5 ppm rates phytoplankton and filamentous algae occurred before the growing season was completed, but no growths were excessive.

Extensive tests on Karmex for the control of filamentous algae were conducted using treatment rates of 0.5, 0.75, 1.0, and 3.0 pounds per surface acre. All rates above 0.5 pounds gave season-long control of filamentous algae which included *Pithophora*, *Spirogyra*, *Oedogonium*, and *Cladophora*. No harmful effects were recorded on zooplankton, bottom organisms or fish, and phytoplankton blooms were reestablished 2 weeks after treatment. At current prices, Karmex will provide filamentous algae control for less than three dollars per surface acre.

GENERAL

An inexpensive recirculating water system was designed and constructed for use in spawning experiments and egg incubation. Water is pumped from a heated reservoir tank through a filter system, into aquaria or hatching units and then returned to the reservoir. Temperature of the water can be maintained automatically at $\pm 0.5^{\circ}\text{F}$. Changing or cleaning of the filters daily is the only maintenance required. Additional aeration may be added if desired.

Six exhibits were prepared for display at fairs and agricultural study days in the area. An estimated total of 43,000 persons viewed the displays during the year.

Numerous field visits were made to fish farms in Arkansas, Missouri, Mississippi, and Louisiana. Recent results of research at the

station and proven techniques were discussed with farmers to help them solve fish cultural problems.

Contracts were awarded to the Con-Ark Construction Company of Conway, Arkansas on May 15 for the construction of a wet laboratory building, an equipment building, and a chemical and oil storage building at Stuttgart. On July 25, contracts were awarded to the A. H. Thomas Construction Company of Camden, Arkansas for the construction of two office-laboratory buildings and the drilling of a domestic water supply well for use at the Kelso site.

Work on the buildings at Stuttgart progressed slowly and construction was only about 90 percent completed on December 25, the contract expiration date. Construction at Kelso has been ahead of schedule with 55 percent of the work completed in the first 40 percent of the contract time.

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SALMON-CULTURAL LABORATORY

Longview, Washington

Roger E. Burrows, Director

HIGHLIGHTS

Hard fat in the diet of chinook fingerlings results in lipoprotein infiltration of the hepatic cells, degeneration of the spleen and hematopoietic organs, and eventual death of the fish.

Reconditioning of water for reuse has proved practicable for maintenance of both fingerling and adult salmon. Maximum loadings of 100 pounds of fish per gallon per minute of freshwater inflow have been maintained.

Differences in the characteristics of blood and body composition of chinook fingerling have been demonstrated as caused by age, growth, and diet.

Differences of 19 percent in body fat at time of release had no effect on the adult survival of chinook salmon.

High stamina lots of chinook salmon fingerlings had a substantially higher return as adults than low stamina lots.

Six million fall chinook salmon eggs have been planted in the Abernathy incubation channel.

The transplanted run of fall chinook salmon to Abernathy Creek produced 8,900,000 eggs.

NUTRITION

1964 feeding trials

The 1964 feeding trials were a continuation of experiments at this laboratory for the purpose of developing nutritionally adequate diets for the propagation of salmon. This year's experiments were designed to test the protein quality of various fish meals with the objective of finding possible substitutes for salmon carcass meal. Following is a list of fish meals tested: chinook salmon carcass (prepared in

1960), chinook salmon carcass (prepared in 1963), herring, turbot, two dogfish meals, sole, rockfish, and tuna.

To briefly review, these fish meals were added to the basal ration which consisted of dried skim milk, cottonseed meal, wheat germ, a vitamin supplement, peanut oil, CMC, and water. The fish meals were added in varying amounts dependent upon their protein content, and the amount of protein in the diet was held constant by adjustment of water content to compensate for changes in the amount of fish meal. All experimental diets were maintained isocaloric at 2,350 calories per kilogram of diet and fed at a protein level of 25 percent.

After 24 weeks of feeding results were as follows:

1. A rancid salmon carcass meal produced poor growth and histological examination of livers from fish fed this meal exhibited definite evidence of liver toxicity.

2. A dogfish meal containing a high urea content produced inferior growth.

3. Diets containing either the turbot or the low-urea dogfish meals produced fish with growth rates equaling those fed the 1960 salmon carcass meal. Herring meal was inferior to salmon meal.

4. In general, the protein quality of fillet-scrap meals (sole, tuna, and rockfish) were inferior to the quality of fish meals prepared from whole carcasses.

5. Fish meals fed in combination did not produce better growth rates than diets containing a single fish meal.

6. This experiment indicates that other fish meals may be substituted for salmon meal in the composite meal mixture. The ability to make such substitutions makes this ration a practical production diet as all portions of the

mixture are in adequate supply. In 1965 the composite meal mixture will be fed in a large scale experiment to production stock at this laboratory.

Effect of type of fat in diet on chinook fingerlings

A feeding experiment was designed to determine the effect on chinook fingerlings of feeding different types of supplemental fat in a diet. This experiment consisted of three groups of fish, each of which was fed a different diet for a period of 26 weeks. One group was fed our standard 25 percent protein, 2,350 calorie meal and peanut oil diet, the second group was fed the meal diet in which beef spleen fat was substituted for peanut oil, and the third group was fed an all-meat diet consisting of hog liver and beef spleen.

Histopathological examination of preserved livers from these fish exhibited marked differences after 14 weeks of feeding. Both the hard-fat group and the meat-fed group were characterized by concentrated amounts of lipo-protein material within the hepatic cells, while conversely the soft-fat group had none of this material. The soft-fat group did have a fair amount of both neutral fat and glycogen accumulated between liver cells. At the end of 18 weeks of feeding, the group of fish being fed the hard-fat diet was randomly split into two lots. The one lot remaining on the hard-fat diet and the other being put on the soft-fat diet to determine if the changes in the livers were reversible.

At the end of 26 weeks of feeding, fish from each diet were examined histologically. Results at this time showed that livers from hard-fat and meat-fed fish had continued to deteriorate and examination of the spleens and hematopoietic portion of the kidneys also indicated deterioration. These abnormalities in the spleen and head-kidney were accompanied by low hematocrit values for these fish. Little or no recovery was noted in the group of fish from which hard fat was deleted. No abnormalities were detected in the group of fish fed standard meal and peanut oil diet.

We have interpreted these results as evidence that fish metabolize hard fats very poorly. As a consequence, these fats infiltrate and accumulate within the liver cells as lipo-proteins. Either consequently or incidentally, deterioration occurred in both the spleen and hematopoietic portion of the kidney resulting ultimately in a marked hematological deterioration including a pronounced anemia. The changes in the livers and spleens appear to be irreversible after they have once become established.

ENVIRONMENTAL FACTORS

Oxygen demand

The objectives of this experiment were to determine the effect of fish size and water temperature on the oxygen requirements of fall chinook fingerlings. To date studies have shown that during normal activity, oxygen requirements of fingerling chinook changed abruptly when the fish approached 5 grams in weight. At 53°F. the oxygen consumption rate of 5-gram fish was 50 percent less than 1-gram fish. From 5 grams to 15 grams, the rate fell, but slightly, and at the end of this period the rate was about 15 percent less than when they weighed 5 grams. Temperature effects were striking. At 63°F. fish weighing 5 grams used three times as much oxygen as at 43°F.

Comparisons of the oxygen requirements determined under test conditions with those of comparable fish held in rearing ponds indicate good correlation under similar activity levels. These data will be satisfactory for projecting the carrying capacity of hatchery water supplies.

The oxygen requirement of adult chinook salmon being held for spawning was determined to be 0.42 parts per million, per gallon per minute, and per pound of fish at 50°F.

Water reclamation

Experiments were conducted to determine the problems associated with water reuse and reclamation and methods of overcoming these problems. A small recirculating system was

which consisted of a fish-holding trough, settling tank to collect the heavier debris and sediment, an aeration tank, and a recirculating pump.

The pump forced the water through an overflow into the aeration tank maintaining an ammonia level of near air saturation levels at all times.

Tests were conducted with chinook fingerlings in creek water at pH 7.3 and in well water at pH 8. In both water supplies ammonia levels increased rapidly during the first 10 to 14 days after which both the ammonia level and the pH began to decrease and before the end of 3 weeks small amounts of ammonia were measured in the reuse system. When the pH dropped to 5.7 the fish began dying. Investigation showed that a nitrifying bacterial culture which utilized the ammonia excreted by the fish had developed in the reuse system. In the nitrification process bacteria oxidized ammonia to nitrous and nitric acid which combined with basic salts to form nitrates. Since neither water nor solids containing basic salts were added to the reuse system, these salts were gradually depleted, thereby decreasing the pH to a level toxic to chinook fingerlings.

In a subsequent test oyster shell was added to the aeration tank to supply calcium carbonate for the completion of the nitrification process. The shell served effectively both as a buffer and as a source of calcium carbonate which maintained the pH at 7.4 or higher. A group of fish held in the reuse system 5 weeks gained their weight and incurred no mortality. Trace amounts of ammonia nitrogen were measured in the system, however, the nitrates built up from practically zero to 88 ppm.

A larger model reuse system including a rectangular circular tank for fish holding was set up to simulate conditions thought to be applicable to large scale operations. Reconditioning facilities were the same as used in previous tests. Supplemental fresh water in an amount sufficient to provide a theoretical complete water interchange once in 12 hours was added to the reuse system to prevent excessive nitrate build-

up. Chinook fingerlings were held in the reuse system for as long as 12 weeks with excellent results. Nitrates remained below 5 ppm. The only difficulty encountered was an outbreak of bacterial gill disease in one group of fish which was successfully controlled by treatment with Lignasan. At the conclusion of the 12-week test, the reuse system was carrying 104 pounds of fish per gallon per minute of fresh water inflow, 10 pounds per gallon per minute is considered maximum without water reconditioning. The only maintenance required was a weekly cleaning of the filter and settling tank.

Large scale tests were conducted utilizing two rectangular-recirculating ponds for fish holding. Water reconditioning facilities were constructed on essentially the same scale as was used in the model reuse system except that two 12-foot by 12-foot filter tanks were used instead of a filter in the aeration tank. The ponds were stocked with 1,200 pounds of chinook fingerlings each and the experiment carried on for 4 weeks. Although weight gains in the reuse ponds were comparable to those obtained in ponds not on reuse, the reconditioning facilities did not remove the metabolic waste products as effectively as desired. It was assumed that a large part of the nitrifying bacterial culture was lost each time a filter was cleaned and the heavy pond loading necessitated frequent filter cleaning. This assumption was confirmed by tests in the small reuse system. The filter was cleaned 5 times in 11 days and after more than 3 weeks of normal operation, the ammonia nitrogen level had not decreased to the low levels measured prior to the frequent cleaning. These results indicated that an additional filter to increase the time interval between cleanings was needed in the large reuse system and that the initial pond loading should be low to allow the nitrifying bacterial culture to become well established in the reconditioning facilities.

The small reuse system was utilized in another test directed toward increasing the efficiency of water reconditioning facilities. A 12-inch layer of crushed rock was placed in the aeration tank and was not disturbed throughout the test period. After 5 weeks a nitrifying bacterial culture had developed on the rock which

effectively held the ammonia level even though the fish were fed three times a week. The reuse system incorporated a circular tank for large reuse.

Adult chinook salmon were held in a recirculating reuse system on a tributary of the Columbia River and transported to the hatchery and held for 4 weeks. No disease occurred. A large number of fish corresponded to the number of fish in the fish which were held while mature. Sperm or egg production and spawning mechanism of the gonads were normal in the water from the Linn County Hatchery and both cohorts were held at the Abernathy Hatchery. Outbreaks of disease were not successfully controlled. The fish turned a chite green.

The reuse system was found to be practical for both juvenile and adult salmon. Further investigation and reconditioning facilities were developed in 1965.

A complete reuse facility, a model for possible use in a hatchery, apparatus combination reference pump. Water was pumped through a chamber aerated. The tank was cleaned when necessary. The tank contained a large amount of water.

The chinook fingerlings were held in ammonia nitrogen indicating tanks.

effectively held the ammonia nitrogen at a low level even though the filter was cleaned three times a week for 3 weeks. It is planned to incorporate crushed rock bacterial beds in the large reuse system for tests during 1965.

Adult salmon were held successfully in a recirculating water reuse system. Spring chinook salmon trapped at Detroit Dam, located on a tributary of the Willamette River, were transported to the Salmon-Cultural Laboratory and held for over 5 weeks before any mortality occurred. Although the time of death of these fish corresponded to peak spawning of comparable fish in the Willamette River tributaries, the fish which were held in the reuse system, while mature, did not freely discharge either sperm or eggs. It appeared that some triggering mechanism that caused final development of the gonads in these exotic fish was missing in the water of the reuse system. Coho salmon from the Little White Salmon River, Washington, and both coho and fall chinook salmon from Abernathy Creek were held to full maturity. Outbreaks of fungus disease on the fish were successfully controlled by treatment with malachite green.

The results of these experiments indicate that the reuse of water after reclamation is practical for both rearing fingerlings and holding adult salmon. Tests directed primarily toward further increasing the efficiency of water reconditioning facilities will be continued during 1965.

A commercially available fish-holding facility, a Min-O-Cool unit, was tested for possible use in water reclamation studies. The apparatus consisted of a fiberglass tank, a combination refrigeration unit and a water circulating pump. Water was continuously recirculated through a charcoal filter and was adequately aerated. The refrigeration unit operated only when necessary to maintain the set temperature. The tank contained approximately 90 gallons of water.

The unit was stocked with 55 chinook fingerlings weighing 3 pounds. During the test ammonia nitrogen levels increased steadily indicating that the charcoal filter was not

removing the metabolic waste products. Within 3 weeks ammonia nitrogen had built up to over 21 ppm, fish began dying, and the experiment was discontinued.

Although the Min-O-Cool did not prove satisfactory for our purposes and will not maintain chinook fingerlings for extended periods, the refrigeration unit operated efficiently and may be of value for chilling water in other apparatus.

DIFFERENCES IN FINGERLING SALMON

Physiological and chemical differences

Samples from all 1964 fall chinook fingerling releases from the Columbia River hatcheries were measured to determine their physiological and chemical differences. These measurements included weight, performance, hematology, plasma composition, body composition, gross pathology and histopathology. Again, differences were found to exist between samples but the significance of these differences remains to be determined.

Effect of growth and diet on fingerling characteristics

The purpose of this experiment was to determine if the blood and body composition of fingerling chinook salmon altered with age, growth, and diet. Growth and diet effects were measured by analysis of the blood and body components of chinook fingerling at 4-week intervals.

It was not our intent to produce comparable diets, but rather to determine if the diet fed would produce differences and to determine if these differences varied with age and growth. The fish were fed exclusively on a meal or meat diet. The fry were hatched from a single female and were reared at a constant temperature of 53°F. The meal-fed fish received a diet containing 25 percent protein and 2,350 calories per kilogram and the meat-fed fish were fed a diet containing 17.5 percent protein and 1,150 calories per kilogram.

The meat-fed lot weighed more than those fed meal during the first 3 months of feeding. Those fed meal gained 81 percent in weight during the second month of feeding while those fed meat gained only 66 percent. Throughout the experiment, the meal-fed group continued to gain more rapidly than those fed meat and at the conclusion of the experiment had an average weight of 31.2 grams versus the 23.4 grams for the meat-fed group. The meal-fed fish had a higher fat deposition and lower body water content. Those fed meat showed lipoprotein deposition in the liver at 18 weeks and pathological examination of the body organs also revealed marked abnormalities in the spleen and hematopoietic portions of the kidney.

The hematology, as measured by percent small cells, hematocrit, and total corpuscular count, showed that these values were affected by growth and diet. When the fish were 3 months old and had received the meal diet for 2 months, the levels of the small blood corpuscles increased to 51 percent. This level is appreciably higher than the average of 21.2 percent as determined for hatchery fish of comparable size and performance. This increase in small cells was due, probably, to the rapid growth of these fish. In the group fed a meat diet, the hematocrit levels fell sharply at the seventh month, reflecting damage caused by the hard fat in the diet. Corpuscular counts in the meat-fed fish dropped from 1.1 million to 980 thousand during the same period. Hematocrits and corpuscular counts for meal-fed fish increased throughout the feeding period.

Physiological measurements showed that the composition of the blood plasma varied due to age and diet. Plasma glucose levels of both groups increased from 50 mg percent to about 100 mg percent in the 8-month period. The plasma cholesterol content of the meat-fed fish increased steadily during the first 3 months of measurement, then dropped sharply, coincident with the pathological condition noted. Total plasma proteins were very similar regardless of diet but electrophoretic patterns of the plasma proteins did show a protein fraction in the meat-fed fish which was not present in the meal-fed group.

This study as conducted indicates that in order to compare the inherent properties of fish, standards must be established for each age and size group. A paper reporting on this experiment in detail is in preparation.

FINGERLING CHARACTERISTICS AFFECTING ADULT SURVIVAL

Effect of differences of body fat in fingerling on adult survival

The 4-year-old returns of two groups of marked fall chinook salmon are now complete. In 1961 these fish were fed two diets to induce differences in fat deposition. At time of release a 19 percent difference in body fat existed between the two groups. The return to Abernathy Creek amounted to .046 percent for the high-fat fish and .039 percent for the low-fat group. No significant difference existed in these returns. Returns for both groups were low, probably due to heavy infestations with metacercariae at time of release and to previous exposure to a virulent furunculosis infection.

Effect of fingerling stamina differences

The ability of fingerling salmonids to swim as measured by the stamina tunnel has been assumed to be a valid determination of condition. In 1962, an experiment was initiated to determine if differences in the stamina of the fingerlings at time of release affected the survival to the adult stage.

In May of 1962, approximately 400,000 fall chinook fingerlings were randomly divided into two lots of 200,000 fish each. One of these lots was reared in six rectangular-recirculating ponds and the other in four 8- by 80-foot raceways. Water was introduced at the rate of 250 gpm in the rectangulars and 375 gpm in the raceways. At time of release the loading rate was approximately 6 pounds per gpm of inflow. In July the two groups were marked, the raceway fish by the excision of the right pectoral and the fish reared in the rectangular ponds by the excision of the left pectoral fins.

On September 19 and 20, 1962, the two lots of fish were released into Abernathy Creek.

At time of release the average weight of the raceway fish was 20.5 grams and of the fish from the rectangular-recirculating ponds, 23.9 grams. Random samples from each group showed performance ratings of 64 for raceway fish and 99 for the samples from the rectangular ponds. These significant differences in stamina between the two groups were imposed only by the pond type; all other rearing procedures including the diets fed were identical.

In 1964 the fish returned as 3-year-olds. The results of this return are shown in the following table:

	No. <u>released</u>	No. <u>returned</u>	% return
High stamina (LP)	181,859	255	0.140
Low stamina (RP)	198,715	170	0.086

Chi Square: 23.3, highly significant difference

In addition, 3 fish marked right pectoral and 2 fish marked left pectoral returned as 2-year-olds in 1963, all were males. The 3-year-old run from both groups approximated a 50:50 ratio of males to females. These data indicate that a 50 percent increase in stamina results in a 60 percent increase in adult survival and underscores the superiority of the recirculating ponds. We can see no justification for further construction of raceway ponds and recommend the conversion of present low-velocity pond types into high-velocity ponds as rapidly as economically feasible.

SEX CONTROL

Method development

The 1962-63 experiments on sex control proved ineffective in altering the sex ratio of fall chinook salmon. The adjustment of the pH of the milt or of the media of sperm passage over a range of from pH 5.8 through 12 was attempted but was unsuccessful in producing sex selective mortality among either the male or female gene-bearing sperms. It was observed that impaired fertilization and coagulation of milt took place at a pH below 6. A second experiment to determine if differentials

in longevity existed between the male and female gene-bearing sperms was also unsuccessful when tested at time intervals after activation of up to 70 seconds. The 70-second interval was well beyond the commonly accepted period of maximum sperm motility after activation, but fertilization above 95 percent was found in all test lots. Future testing at time intervals of more than 70 seconds was indicated.

The 1964-65 sex control experiment now being conducted was changed to lengthen the time period between activation of the sperm and fertilization of the eggs. Aliquots of sperm activated by the addition of ovarian fluid were withdrawn at minute intervals up to 8 minutes and used to fertilize experimental lots of eggs.

Two additional studies are being made using sperm stratification time as the variable. Tests with rabbit sperm have demonstrated that differences in density can be used to separate the male and female gene-bearing sperms. The technique employed was to place pooled sperm samples from several male chinook salmon in a series of Nessler tubes. After the tubes of sperm were allowed to stratify for fixed time intervals, the top and bottom 10 percent of sperm from each tube were withdrawn and used to fertilize separate experimental lots of eggs. Sexing of the resultant fingerlings will determine if stratification of the sperm has occurred. The first study is testing the effectiveness of sperm separation at 10-minute time intervals up to 1 hour. The second study is testing separation at 24-hour intervals for up to 4 days to measure the effect of longer stratification periods.

All test lots have hatched and the resulting fry should be large enough for sex determination shortly after the first of the year. High mortalities at hatching occurred in many groups of eggs on the stratification experiments after 50 minutes of stratification. This was especially true of those groups fertilized with the sperm from the bottom of the Nessler tubes.

ABERNATHY CREEK INCUBATION CHANNEL

The 1963-64 portion of a project to evaluate the effectiveness of the incubation channel in establishing a significant run of chum salmon was completed in June. Migration of fry from the channel produced survivals of 91.7 percent for the 160,000 eyed eggs of Abernathy stock and 81.7 percent for the nearly 800,000 eyed eggs from the Quilcene National Fish Hatchery. The eggs were planted at a rate of 400 eggs per square foot of gravel used. These survivals compare favorably with previous experiments of the Bureau of Commercial Fisheries where eggs planted at a maximum rate of 140 eggs per square foot of gravel had survivals ranging from 75.0 percent to 95.5 percent.

The objectives of the 1964-65 study are (1) to determine the capacity of the channel in the incubation of fall chinook eggs and (2) to determine the significance of the contribution from surplus fall chinook eggs planted in the channel to the adult return. Approximately 4,000,000 eyed eggs from chinook salmon returning to Abernathy Creek were planted in the channel during October 1964. In addition, 2,000,000 eyed eggs were received from the Little White Salmon National Fish Hatchery and planted in the channel in mid-November. These eggs were planted at the rate of 435 eggs per square foot or nearly 9 percent higher than the stocking rate used in 1963-64. The resulting fry will be compared with 5-month and 7-month-reared hatchery fish on the basis of adult returns. Fry from the channel will be unmarked. All hatchery fish will be marked either by the feeding of tetracycline drugs or by fin clipping. Over 13,000 fry had migrated from the channel by the end of December.

The adult chum salmon returns from both native stock and a 230,000 fry release in 1961 was insignificant and did not provide enough eggs for experimentation. A total of 12 males and 15 females returned providing only about 44,700 eggs. Two of the adult fish had been fin clipped with one of the two marks used on the 1961 channel-reared fry indicating that they were early migrants from the channel. About 40 percent of the fry released were marked either as early or late migrants.

GENERAL

Two groups of fingerling fall chinook salmon were released into Abernathy Creek in 1964. The first group consisted of 630,700 fingerlings weighing 10,520 pounds and averaging 60 per pound were released in May. The second release was made in August and consisted of 205,700 fish weighing 15,785 pounds and averaging 13 per pound. This release contained two marked lots which were debilitated due to diet. All releases were derived from the 1,100,000 eggs taken from Abernathy Creek chinooks in the fall of 1963.

The 1964 run of adult fall chinook salmon consisted of 3,611 fish. Of these, 2,138 fish entered the holding pond and 1,473 spawned in the 1-1/2-mile stream section below the diversion weir. Aging of the fish is not complete but it is obvious that 3-year-olds dominated the run. This 1961 year class was also dominant in the 1963 run.

The total egg take was 5,663,000 with an average of 5,007 eggs per female. After eyeing in the incubators 3,949,000 were planted in the incubation channel and 1,435,000 eyed eggs were retained for artificial propagation. Loss to eye on the entire lot amounted to 4.9 percent. Total loss to fingerling on the hatchery-reared fish has amounted to 8.2 percent.

Of the 1,473 fall chinook spawning naturally in Abernathy Creek, 641 were females with an egg potential of 3,205,000. This number of eggs is well beyond the capacity of the spawning area available and the capacity of the stream to support. Superimposition of redds was common and collection of displaced eggs in the pools was obvious. To further aggravate the situation the creek has experienced two periods of high water while the eggs and fry were in the gravel. We are preparing to fyke net the creek above its confluence with the incubation channel to arrive at an estimate of natural survival.

The alteration of the raceway ponds to the rectangular-recirculating type was completed in time to receive the first feeding fingerlings of the 1964 brood. In order to accomplish this it was necessary to delay development in the fry

by incubating them on the colder creek water. Flow patterns and fish distribution in the altered ponds are excellent.

The staff attended the Northwest Fish-Cultural Conference in December. Combs, Elliott, Fowler, Thomas, and Burrows presented reports reviewing the results of recent research.

STAFF

	Roger E. Burrows, Fishery Biologist
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SOUTHEASTERN FISH CULTURAL LABORATORY

Marion, Alabama

Kermit E. Sneed, Director

HIGHLIGHTS

Effects of fat and carbohydrate on the growth of channel catfish fingerlings are being investigated.

Preliminary research was initiated on the method of binding and transport of I^{131} in the blood of channel catfish, white catfish, gars, buffalofishes, suckers, and rainbow trout.

Cooperative research has been established with personnel of the Alabama Medical College and the Veterans Administration Hospital in Birmingham.

Eight additional catfish hybrids were produced.

Research was conducted *in-vivo* and *in-vitro* on rate and amount of accumulation of $Na I^{131}$ in tissue of fishes.

Most I^{131} activity in eggs from $Na I^{131}$ -injected channel catfish has been found in the egg shell and/or chorion.

NUTRITION OF CATFISHES

The amino acid test diets used in our first experiments, 1962, were nearly identical to those fed to salmon and trout in similar experiments. However, while trout and salmon demonstrated a measurable rate of growth on the amino acid-complete diet, channel catfish lost weight. During subsequent summers, we tested different diet binders, types and levels of carbohydrate and fats, ratio and levels of amino acids, levels of bulk and content of water in the diet. In 1962 and 1963 we observed that ingested food passed through the stomach and small intestine within minutes and concluded that the failure of the fish to grow was due to the nutrients passing sites of absorption before any significant amount could be absorbed. We postulated that the level of magnesium ions and sulfate ions in the salt mixture was high enough to act as a cathartic. A low magnesium-ion and

sulfate-ion mineral mixture was included in several different amino acid test diets and whole protein test diets. At the conclusion of a 4-week experiment, channel catfish fingerlings that were fed the whole protein diet increased 60 percent in weight, while fish on all the amino acid test diets decreased 5 to 15 percent in weight. (This tends to discount the catharsis hypothesis).

Effects of fat and carbohydrate on the growth of channel catfish fingerlings were investigated. Agar-bound purified diets that contained three fats (liquid corn oil, hydrogenated corn oil or beef tallow) at three levels (3, 6, or 11 percent dry weight basis) and one carbohydrate (white dextrin) at two levels (12 or 22 percent dry weight basis) were fed to channel catfish fingerlings in aquaria. Proximate analysis of the tissues and iodine numbers of the stored fats are being determined.

CHARACTERISTICS OF BLOOD OF FISHES

Preliminary research was conducted on the transport of I^{131} in the blood of channel catfish, white catfish, gars, buffaloes, suckers, and rainbow trout, *in-vivo* and *in-vitro*. The degree of I^{131} retention by the blood proteins (*in-vivo*) appears to vary with species and with physiological condition (retention is greater in diseased, starved fish than non-diseased, well fed fish).

In-vitro studies indicate that the iodine uptake by proteins is under enzymatic control: 2, 4-dinitrophenol, an energy blocking compound, when added before the $Na I^{131}$ prevents iodide uptake by the serum proteins; however, iodide uptake by the serum proteins occurs if the 2, 4-dinitrophenol is added after the serum and $Na I^{131}$ have had time to react. Thiouricil also will prevent the uptake of I^{131} by the blood proteins.

In-vitro research indicates that the serum of channel catfish does not "bind" the iodide to the globulins or albumins when these are extracted separately. However, it appears that about 5 percent of the iodide is bound to some protein

since a trichloroacetic acid precipitate retains 5 percent of the total activity even after being washed nine times.

In August we initiated two new cooperative experiments with the personnel of the University of Alabama Medical College in Birmingham. One concerns immunization of fishes and the other involves the identification of components of fish bloods.

STUDIES ON CATFISH HYBRIDS

We continued studies on hybridization techniques for catfishes. In addition to the 6 hybrids produced in 1963, we produced 8 different groups to bring the total number of hybrids to 14. The hybrids produced in 1964 are the brown bullhead X yellow bullhead, flathead catfish X white catfish, channel catfish X brown bullhead, flathead catfish X yellow bullhead, black bullhead X yellow bullhead, white catfish X yellow bullhead, blue catfish X yellow bullhead, and channel catfish X yellow bullhead.

The blue catfish X channel catfish hybrid (produced in 1963) exhibits excellent growth and secondary sexual characteristics far in advance of fish of either parent species of the same age.

METABOLISM OF IODINE IN FISHES

Samples of skin, muscle, spleen, bladder, conus arteriosus, retina, and testes (anterior)

from mature male channel catfish were incubated with Na I¹³¹-Ringer's solutions. At the end of 24 hours, all the tissues except the testes had higher I¹³¹ activities than the corresponding Na I¹³¹-Ringer's bathing solutions. At the end of 48 hours, the retinal tissues had six times the I¹³¹ activity of the bathing solution.

A summary of a typical *in-vivo* experiment with sexually mature male and female channel catfish is tabulated at the bottom of this page.

The data demonstrate that the maturing ovary has sufficient iodide demand to draw iodide from other tissues. No comparable demand is demonstrated by any of the tissue from the male fish. The thyroid was not included due to the near impossibility of obtaining an accurate weight or representative sample of this diffuse tissue.

In cooperation with the personnel of the Veterans Administration Hospital in Birmingham the metabolism of iodine in the ovary of fishes was studied. In both *in-vivo* and *in-vitro* experiments with intact, unspawned eggs of channel catfish, some of the I¹³¹ appears to be bound by the proteins. Numerous washings of the macerated eggs with acetone alone and with acetone plus "cold" Na I did not remove all the activity (I¹³¹) from the egg proteins.

Spawned eggs from Na I¹³¹-injected female channel catfish have a very high level of

Percentage distribution* of I¹³¹ in the tissues of sexually mature channel catfish after intraperitoneal injection with Na I¹³¹.

Tissue	Hours between injection and sacrifice					
	24		48		120	
	Male	Female	Male	Female	Male	Female
Whole blood	18	6	19	2	11	1
Head kidney	6	2	8	1	4	T
Kidney	6	3	8	1	4	T
Gonad	29	45	13	83	16	92
Skin	9	4	10	1	6	T
Muscle	1	T	2	T	1	T
Bile	14	8	16	7	28	5
Eye	5	3	9	1	7	T
Urinary bladder	12	29	15	4	23	2

*Based on I¹³¹ concentration per gram of each tissue

I^{131} activity. Practically all this activity is in the egg shell and/or chorion. Acid hydrolysates prepared from the eggs or shells indicated no free I^{131} . Analysis of the organically bound I^{131} by paper chromatography indicates that the iodinated compound could be monoiodotyrosine (MIT).

Mr. Robert Tarrant, Fishery Research Biologist, joined our staff in October. A Master of Science graduate of Southern Illinois University, Mr. Tarrant spent the last 4 years with the Bureau of Commercial Fisheries conducting research on the physiology of salmon. Since coming to Marion, he has begun a preliminary study on olfactory abilities of channel catfish.

An office-laboratory building and a service building, completed in late 1963, were dedicated in March 1964. These buildings were

a welcome addition to the Southeastern Fish Cultural Laboratory. In the near future, we hope to secure a new "wet" laboratory and pond development to complete our research facilities.

Personnel of the laboratory are directing the research of two of the students of the Marion In-service Training School. One of the problems concerns plant nutrient supplementation of well waters and the other involves suppression of reproduction with synthetic steroid compounds in feeds.

STAFF

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Clara Daniel, Biological Aid
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PESTICIDES

FISH-PESTICIDE RESEARCH LABORATORY

Denver, Colo.; Jackson and Beulah, Wyo.; Willard and Longview, Wash.; Tishomingo, Okla.; Patuxent, Md.; Marion, Ala.; La Crosse, Wis.; and Sandy Hook, N. J.
Oliver B. Cope, Director

HIGHLIGHTS

New investigations were initiated at five existing Division laboratories; fish-pesticide studies are now being undertaken at 10 locations.

Design of a new fish-pesticide laboratory for Columbia, Missouri, was almost complete at the end of the year; well drilling at the site was completed.

Exposures of cutthroat trout to malathion for 2 years resulted in consistent patterns of cholinesterase inhibition and recovery.

Exposures of bluegills to Mirex resulted in small increases of treated fish over the control for average fish size, hematocrit, and serum protein.

Exposures of bluegills in pools to sodium arsenite caused pathology in kidney, liver, and ovary of the fish and reduced bottom fauna.

Casoron residues accumulated in warm-water fish, but no other effects on fish were noted from sublethal treatments.

Measurements of acute toxicities of insecticides, herbicides, and other pesticides were made for a variety of fish species at Denver, Patuxent, and LaCrosse.

Measurements of acute toxicities of many pesticides were made for fresh-water invertebrates at Denver and for estuarine invertebrates at Sandy Hook.

Facilities were ready at year's end for initiation of selective breeding of rainbow trout for resistance to DDT at the Fish Genetics Laboratory.

Studies on intermediary metabolism in salmonids began at the Western Fish Nutrition Laboratory.

PEST CONTROL PROGRAMS

The U.S. Forest Service applied an experimental airplane treatment of 1/4-pound per acre of Cygon (dimethoate) in July to test its utility for spruce budworm control. The 1,000-acre plot was located on the South Fork of Iron Creek in the Salmon National Forest in Idaho. The spray pattern was complete, and the toxicant reached the streams in the area. We measured no effects on fish or aquatic invertebrates at the sampled stations.

EXPERIMENTAL FIELD STUDIES

Malathion and cutthroat trout at Jackson, Wyoming

In phase II of the experiment, involving feed and bath exposures of adult cutthroat to malathion, brain cholinesterase activity followed a definite pattern throughout the year. Control fish exhibited a steady decline in specific activity with increase in size at about half the level reported for fingerlings when the experiment began in February 1963. After each treatment, brains of treated fish displayed declines in activity, followed by recovery over 30-day periods. The fish exposed to the most malathion sustained the most inhibition throughout the experiment. Fish showing the greatest decline in the November 1964 treatment had cholinesterase activities that were 41 percent of those of the control fish.

No significant differences among lots have yet appeared for growth rates or for day-to-day mortality. Histopathology studies show liver lesions appearing within 2 days after exposure and disappearing in 30 days. Changes seen in cholinesterase activity have little permanent physiological significance, according to Dr. Wood's cytologic evaluation.

Mirex and bluegills at Marion, Alabama

Mirex is an insecticide with low acute toxicity for fish and high efficiency as an ant killer and is widely used in the Southeast. A feeding experiment and a contact experiment were conducted at Marion with bluegills and Mirex.

The feeding experiment featured weekly rations of Mirex in the diet of 13-gram bluegills in plastic pools. Each concentration, 5.0, 3.0, 1.0, and 0 mg/kg was fed weekly in two ponds. When the experiment was terminated in December no differences among lots were seen in total serum protein, electrophoresis patterns, microhematocrit, length of fish or weight of fish. Whole-body residues of Mirex were highest in fish fed 5 mg/kg, averaging 3.39 ppm at 14 days; fish fed 3 mg/kg had 3.01 ppm, and fish fed 1 mg/kg had 1.05 ppm.

Contact exposures of bluegills to Mirex in earthen fish ponds had two ponds treated at 1 ppm, two ponds at 0.0013 ppm and one pond as control. When the ponds were drained in November, fish with smallest average size, lowest hematocrit percentage, and lowest serum protein values were found in the control pond, but differences may not be significant. Residues of Mirex in fish, through the 7-day samples, showed highest accumulations in the highest-treated ponds. Rate of storage was extremely rapid in all treated fish. Residues of Mirex in bottom muds were lower than in the fish but were much greater in the aquatic vegetation. Numbers of aquatic invertebrates sampled with plate samplers and dredges were approximately the same in all ponds.

Sodium arsenite and bluegills at La Crosse, Wisconsin

The study initiated in 1963 at La Crosse, featuring long-term sublethal exposures of bluegills to various amounts of sodium arsenite, was continued in the laboratory in 1964, with work on residue analyses, histopathology, processing bottom fauna and plankton, and assembling data for preparation of a report.

Analyses for residues of arsenic in whole fish showed accumulations in all fish. Adults and immatures stored arsenic at about the same rates, and heavier the treatment, higher the residues. Residues in organs of fish from a group that contained whole-body residues of 2.61 ppm at 16 weeks were: flesh, 1.36 ppm; skin and scales, 2.41; gills and digestive tract, 17.60; liver, 11.60; kidney, 5.89; ovaries, 8.39.

Arsenic residues in water collected at 16 weeks were correlated with treatment level, the largest being 9.04 ppm in the pond treated once a week at 0.69 ppm. In bottom soils, the same pattern existed, with 109.9 ppm in the pond of heaviest treatment. Disappearance of arsenic from water and soils was more rapid at low than at high treatment levels.

No pathology appeared in fish in the first few weeks, but kidney and liver damage appeared thereafter along with degenerative lesions in the ovaries. Nematodes whose incidence was high in the pyloric caecae at the beginning of the experiment disappeared after 2 weeks of exposure.

There was a reduction in bottom fauna in heavily treated ponds, with numbers of organisms per sample amounting to less than half of those in the control and lightly treated ponds, and there was a trend toward reduction of numbers of species with increase in concentration of sodium arsenite. Plankton samples showed depression of numbers of rotifers, cladocera, and copepods, especially in heavily treated ponds.

Diquat and bluegills at La Crosse, Wisconsin

An experiment to measure chronic effects of the herbicide Diquat to adult and immature bluegills was carried on from May to August at La Crosse. Concentrations of 1.0 and 3.0 ppm of Diquat were used at various frequencies.

Samples of fish, water, plankton, bottom organisms and mud were collected at intervals. Fish are being studied for residue content, pathology, and changes in blood constituents.

Water is being analyzed for Diquat residues, and plankton are being counted. Bottom organisms apparently were not reduced in numbers by Diquat.

Survival of adult and immature bluegills was not affected by Diquat. Growth of immature bluegills was not influenced by the herbicide, but adults had slightly less weight gain in treated pools than in the controls.

Casoron and bluegills at Tishomingo, Oklahoma

The herbicide Casoron (dichlobenil) was studied at Tishomingo with respect to long-term effects on bluegills. The weed killer was applied in earthen fish ponds at rates of 40, 20, 10, and 0 ppm as a wettable powder. Mortality up to about 25 percent occurred immediately in heavily treated ponds but was lighter in other treated ponds.

Residues of Casoron in the waters declined rapidly after the 3-day sample, and only minute amounts were found in the water at 85 days. Concentrations of Casoron in bottom sediments at 7 weeks were 0.2 ppm or less. Regrowth of aquatic vegetation was inversely proportional to strength of treatment.

When the experiment was terminated in the field, the largest fish were found in one of the ponds treated at 40 ppm. At this time, immatures were found in all ponds, with smallest numbers associated with high treatment.

Casoron and warm-water fish at Denver

A study of Casoron, bluegills, small-mouth bass, yellow perch, and green sunfish was made in one small pond near Denver. The formulation was granular, at 10 pounds per acre (0.6 ppm), in one application.

Residues in the water reached a peak at 16 days (0.32 ppm at the surface and 0.54 beneath the surface), and there was still 2 ppb at 166 days.

Exposure to Casoron at this level did not result in mortality to the fish. Residues of the

herbicide were measured in all four species; bluegill, yellow perch, and green sunfish generally accumulated greater residues than did black bass, but all species had accumulated 4 - 8 ppm at 34 days.

Paraquat and fish at Denver

The herbicide Paraquat was studied in a small pond near Denver to measure persistence and chronic effects on rainbow trout, green sunfish, bluegills, and channel catfish. Treatment was at 1 ppm; the peak of residue in the water was during the first day, and no Paraquat was detectable at 32 days.

No acute toxicity to fish was seen. Residues developed in all fish measured, with peaks of accumulation seen from 1 to 16 days, depending upon the species. No pathology attributable to Paraquat has been seen in 40 specimens examined so far.

LABORATORY STUDIES

Fish toxicity tests at Denver

Bioassay work at Denver included preliminary testing of new pesticides against rainbow trout and bluegills, studies on the herbicide Treflan, time-temperature studies on rainbow trout and bluegills with several insecticides, and special studies on DDT and the New Zealand strain of rainbow trout.

Concentrations that killed 50 percent (LC₅₀) of 40 - 56 mm rainbow trout at 55°F. were determined at 24, 48, and 96 hours for 36 insecticides, for 14 herbicides, and 4 other pesticides. For bluegills 46 - 48 mm, at 75°F., determinations were made for 17 insecticides, 21 herbicides, and 4 other pesticides.

Tests on influence of time and temperature on toxicity, covering 24 - 96 hours and 35° - 65°F. for trout and 45° - 85°F. for bluegill, were conducted with: DDT, toxaphene, and malathion for rainbow trout; endrin, trifluralin, dieldrin, and lindane for bluegills.

The herbicide Treflan was the subject of special studies because of its extreme toxicity to

n, compared with other herbicides. Bioassay tests were conducted with Treflan and rainbow trout and bluegills, and outdoor plastic pools were used to measure toxicity when the herbicide is in contact with soil. Introduction of the herbicide in a soil slurry at one pound per acre resulted in mortality as serious as that seen when no soil was present, although it is claimed that Treflan is bound to soil particles under the conditions of recommended application. Bioassay work on DDT and rainbow trout of the New Zealand strain was performed to reach an understanding of susceptibility, preliminary to establishment of treatment levels to be used in selective breeding studies at the Fish Genetics Laboratory. The New Zealand strain appears to respond to DDT in the same way as other rainbow trout tested.

Fish toxicity tests at Sandy Hook

Testing of seven organochloride and two organophosphorous insecticides against a variety of fishes, grass shrimp, and mud snail was carried forward in the Sandy Hook Marine Laboratory. Effects of salinity and temperature on toxicity were measured on these estuarine animals. It was found that higher temperatures accelerated mortality for the toxicants tested, except that mummichogs, Fundulus heteroclitus, were most sensitive in the lower range - 68° to 77°F. Mummichogs were most sensitive at intermediate salinities, eels had increased sensitivities with increased salinity, grass shrimp showed decreases in mortality with increased salinity. Mummichogs exposed to LC₇₅ (24-hour) amounts of chlorinated hydrocarbon insecticides for periods of 24 hours to 48 hours showed negligible mortality during a 21-day observation period after being exposed less than 2 hours, relatively low mortality resulted from exposures of about 24 hours, and almost complete kill with exposures of 48 hours or longer.

Adult northern puffers, Sphaeroides lineatus, were exposed to graded concentrations of endrin at 20°C., 24 o/oo salinity, and 18.0. A concentration of 10 ppb of endrin killed all test animals in 24 hours but none died at concentrations of 1 ppb or lower.

Blood and tissue chemistry of survivors were examined by photoelectric colorimetry and atomic absorption spectroscopy. Mean hemoglobin content and relative liver size of puffers exposed to 1.0, 0.5, and 0.05 ppb endrin were not significantly different from those of controls. Serum concentrations of Na, K, Ca, and cholesterol were consistently higher in experimental animals than in controls, but no obvious trends were noted in levels of serum chloride, gamma-globulin, and uric acid.

Concentrations of Na, K, Ca, Mg, and Zn in liver of test animals were consistently lower than those of controls; concentrations of the same cations in gill tissues fluctuated widely.

It is concluded that exposure to sublethal concentrations of endrin resulted in liver damage as evidenced by accelerated transfer of major cations and cholesterol from hepatic tissue into serum with increasing concentration of endrin.

Fish toxicity tests at Patuxent

The Fish-Pesticide Research Laboratory substation at Patuxent, Maryland, conducted studies on the effects of water hardness on the toxicities of herbicides to fish and tested new herbicides for toxicity to four species of fish.

In extensive tests on water hardness, it was concluded that water hardness did not significantly influence toxicity for the combinations tested, except in the case of silvex and black bullhead; here, toxicity in the hardest water was double that in the softest water.

Invertebrate toxicity tests at Denver

Bioassay with immature aquatic insects and pesticides at Denver resulted in an enlargement of our catalogue of toxicities in the laboratory situation. During the year we established LC₅₀ values for 24, 48, and 96 hours with 19 insecticides and 12 herbicides for the stonefly, Pteronarcys californica, and numerous insecticides for the mayfly Baetis, the stoneflies Isoperla and Claassenia, the damselflies Ophigomphus and Amphagrion, the snipefly Atherix, the crane fly Tipula, and a caddisfly of the family

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Limnephilidae. Some work was also done with Planaria and Daphnia. The order of toxicity for these invertebrates is very different than that for fish, and time is more important with aquatic invertebrates than for fish.

DDT and rainbow trout at Beulah, Wyoming

Testing, holding, and spawning facilities have been assembled in preparation for proposed new studies to measure inherent and acquired changes in susceptibility of New Zealand strain rainbow trout to DDT. Preliminary DDT toxicity measurements with the New Zealand strain were made at Denver and Beulah.

Intermediary metabolism studies at Willard, Washington

A beginning was made at the Western Fish Nutrition Laboratory at Willard on studies in intermediate metabolism in salmonids. Three staff members are on duty, and have begun work on normal metabolism prior to measuring effects of exposure to pesticides.

Chemical methods development

Chemists at Denver and Patuxent made improvements in the methods of chemical analysis of residues of various pesticides in water, mud, fish, and aquatic vegetation. Noteworthy were results with Casoron, Paraquat, 2,4-D, and dimethoate, all of which required important modifications of published methods for analysis of residues in other kinds of materials.

GENERAL

Progress was made in the design of a fish-pesticide laboratory for Columbia, Missouri. Region III engineers had almost completed detailed design work at the end of 1964. Well drilling was completed at the laboratory site, and the predicted flow of water is available.

Five new substations for pesticide work were established at existing Division laboratories. Staffings and development of facilities went forward at the Western Fish Disease Laboratory, Western Fish Nutrition Laboratory, Salmon-

Cultural Laboratory, Sandy Hook Marine Laboratory, and the Fish Genetics Laboratory.

Personnel engaged in training in Leadership, Statistics, Gas Chromatography, Biochemistry, Oceanography, and Aquatic Biology.

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CONTROL

FISH CONTROL LABORATORIES

La Crosse, Wisconsin; Warm Springs, Georgia

Robert E. Lennon, Director

HIGHLIGHTS

Priority research was accomplished on fish anesthetics which require clearance by the U.S. Food and Drug Administration.

Toxicity of 22 disease treatment compounds to lake trout and bluegills was determined.

Intensive tests of antimycin A (Ichthyocide) in the laboratory and field furthered its development as a fish toxicant.

A four-man bioassay team of the Fish-Pesticide Research Laboratory was established at La Crosse.

CHEMICAL SCREENING

Preliminary and delineative screening

Selected nitro-salicylanilides were screened against rainbow trout and goldfish. All were active and killed fish rapidly at low concentrations. The fish evidenced a high degree of irritation upon exposure which was followed by unusual voiding of mucous.

Nitro-salicylanilides are an especially interesting class of chemicals because of their intense activities against fish. Some are selectively toxic; others are not. It seems that activity is related to substituted active groups on the parent structure. Position of a substituted group on the structure influences toxicity and in some instances determines selectivity of the compound. Relationships between structure and activity have been under limited investigation to develop leads for selective control agents.

Several organic solvents which are used routinely in preparing chemicals for bioassays are undergoing trials to detect their toxic or other effects on fish. Acetone has been tested

against 10 species and ethanol against 2 species at 12°C. Findings indicate that bluegills are considerably more sensitive to acetone than green sunfish, longear sunfish, and pumpkin-seeds. Also, acetone at 4 to 8 parts per thousand causes stress in most species. These data are especially important when large amounts of acetone must be employed to dissolve the more insoluble test compounds. Care is taken to keep aliquots small to prevent the solvent from enhancing the activity of a test compound.

Extensive testing of p,p'DDT as a reference toxicant against various lots of routine bioassay fishes was nearly completed. Results are not entirely satisfactory because of the extreme resistance of some fish. Goldfish, for example, are highly resistant and the toxicant acts too slowly on them to produce meaningful mortalities in less than 96 hours. Also, we have been unable to determine the concentration lethal to 50 percent (LC₅₀) for bluntnose minnows because of inconsistent mortalities at progressive concentrations. Specimens became extended with gas and withstood much higher concentrations than other species. In contrast, largemouth bass and yellow perch are among the more sensitive. Upon completion of the study a decision will be made whether to continue use of p,p'DDT as a reference toxicant or seek a substitute.

Much of the work planned for the preliminary and delineative projects was deferred in order to concentrate efforts on fish anesthetics and disease treatment compounds and to assist in intensive screening of antimycin A as a fish toxicant. Employment of student help during the summer and autumn accelerated the pace of bioassays greatly.

At a meeting with representatives of the U.S. Food and Drug Administration in March, we elected to see that the research necessary to clear MS-222, Quinaldine, and methyl pentynol as anesthetics for fish is done. A new anesthetic,

McN-JR-7405, was included later. The studies are divided into three phases: (1) toxicity of the chemicals to fish; (2) their efficacy as anesthetics; and (3) their residues in fish tissues. Work on phases 2 and 3 was assigned to the physiology project. Cooperation of industry in all phases is sought.

Observations on toxicity of MS-222, Quinaldine, and methyl pentynol to nine species of game fish of two or more sizes are nearly complete. McN-JR-7405, a more powerful compound, has been tested against three species. In general, lake trout are more susceptible to anesthetics and channel catfish are more resistant. Also, small fish are more susceptible than large ones. Trials have demonstrated advantages and disadvantages in each of the chemicals. Quinaldine, for example, will be tested further as a possible collecting tool. Methyl pentynol is less effective than the others as an anesthetic, but it may have an advantage as a distribution aid despite its distinctly unpleasant odor.

The Eastern Fish Disease Laboratory at Leetown requested cooperation in determining the toxicity of certain disease treatment compounds to fish. Tests with lake trout and bluegills are complete (table 1). The specimens were 1 to 3 inches long, and assays were conducted in accordance with our regular procedures. Tests with other species are in progress. In some cases, saturated solutions of compounds did not cause mortalities of fish, and they were not tested further. The most toxic of the compounds is malachite green which killed fish at less than 0.6 ppm within 24 hours. In contrast, formaldehyde is relatively non-toxic.

Intensive screening

Antimycin A progressed rapidly in development as a fish toxicant, and Ayerst Laboratories, Inc. has registered it as Ichthyocide. We concentrated efforts on it in the laboratory and field and most studies are complete or nearing completion.

Laboratory:--The concentrations of Ichthyocide effective on none, half, and all fish (EC_0 , EC_{50} , and EC_{100}) were determined for

18 species during the year. This raised the total species used with the toxicant to 28. Among them, salmonids and percids continue to demonstrate greater sensitivity and catfishes show greater resistance. In another phase of experimentation, effective lethal contact periods for Ichthyocide against selected species were observed. Fish exposed to this toxicant tend to die slowly, and it was suspected that they have had lethal exposures long before they exhibit symptoms of distress or death. Carp, for example, which were subjected to 10 ppb of Ichthyocide for 2 to 4 hours before removal to fresh water went on to perish even though death occurred hours later. Specimens exposed for 30 to 60 minutes survived. Moreover, specimens which were given lethal exposures and then removed to running water showed interesting symptoms of distress preceding death. They were: negative rheotaxis, schooling, negative response to light and touch, and loss of equilibrium. These data are important to possible use of the toxicant in streams.

Ichthyocide was tested in waters of different qualities and pH. It was slightly less effective against rainbow trout, goldfish, and bluegills in reconstituted waters with hardnesses of 20 to 400 ppm. Results indicate that it degrades rapidly in alkaline waters, and the rate is more rapid at increased temperatures.

Eggs of rainbow trout, white sucker, and northern pike were exposed to various concentrations of antimycin for short lengths of time. Thirty-minute exposures to 0.1ppm of antimycin killed all eggs whereas smaller concentrations permitted some survival. Comparable trials with the chlorinated hydrocarbon, Thiodan, caused no mortalities in eggs.

An investigation was begun on chemicals which might be used easily and economically to detoxify antimycin in lakes and streams. Potassium permanganate at 500 ppb effectively detoxified 5 ppb of antimycin.

Plastic wading pools:--Extensive experiments were run with concentrations of Ichthyocide up to 200 ppb against various species and sizes of fish, in warm and cold waters of differing hardnesses and pH, and in the presence of aquatic

Table 1:--Concentrations of fish treatment compounds (in p.p.m.) which produce 50-percent mortality (LC₅₀) in selected species of fish at 12° C.

Compound	Lake trout		Bluegill	
	time in hours		time in hours	
	24	48	24	48
Acriflavine	37.5	28.0	18.0	13.5
Amopyroquin	15.5	14.0	33.0	18.5
CoRal	6.8	4.0	10.5	8.0
Erythromycin thiocyanate ^{1/}	>100.0	>100.0	>100.0	>100.0
Flagyl ^{1/}	>100.0	>100.0	>100.0	>100.0
Formaldehyde	220.0	167.0	135.0	140.0
Malachite green	0.6	0.4	0.3	0.1
Merthiolate	13.0	2.1	110.0	64.5
Methylene blue	35.0	34.0	51.0	33.0
Neguvon	41.0	9.0	78.0	71.0
Nickel sulfate	170.0	75.0	>240.0 ^{1/}	>240.0 ^{1/}
P.M.A.	12.5	7.6	20.0	16.0
Quinacrine HCL (atabrine)	28.0	21.0	120.0	79.0
Quinine HCL ^{1/}	>100.0	>100.0	>100.0	>100.0
Roccal	2.7	2.0	2.1	1.7
Ruelene	27.0	27.0	36.0	35.0
Tiguvon	6.5	5.3	15.7	8.9
Trolene	0.7	0.6	2.1	1.0
TV-1096	32.0	16.5	>100.0 ^{1/}	>100.0 ^{1/}
Sulfamerazine ^{1/}	>100.0	>100.0	>100.0	>100.0
Sulfamethazine ^{1/}	>100.0	>100.0	>100.0	>100.0
Sulfisoxazole ^{1/}	>100.0	>100.0	>100.0	>100.0

^{1/} No mortality recorded at the indicated concentration

invertebrates and plants. Among the results, black bullheads showed an ability to survive concentrations which caused them to exhibit distress. This was totally contrary to the behavior of other species. Also, they survived 200 ppb of antimycin in water of 80°F. and pH 9, but succumbed to 160 and 200 ppb at 73° and pH 7.8 to 8.6. Concentrations of 5 ppb were effective in killing all other fish, and there were no harmful effects on damselfly naiads or vegetation. A series of tests in November at 35°F. proved that antimycin is slightly less toxic at low temperature.

Field:--Ichthyocide was applied in a small stream in cooperation with the Wisconsin Conservation Department. The experiment was successful and showed where attention must be focused in later trials. There was no evidence of the toxicant repelling fish or driving them downstream. An assessment of the kill was accomplished by electrofishing.

A new formulation of Ichthyocide was employed against 17 and 19 species of fish in ponds at the Berlin, New Hampshire and Cape Vincent, New York National Fish Hatcheries, respectively. It was more effective and persisted longer in the cold and soft water containing 10 ppm total dissolved solids (TDS) at Berlin than at Cape Vincent (160 ppm TDS). The six ponds at the two hatcheries totalled 10.4 acre-feet, and only 59 grams of antimycin were used to treat them. Concentrations of 1.2 and 12 ppb killed all fish except brown bullheads at Berlin; 10.4 ppb killed all fish and 3.1 ppb killed most fish at Cape Vincent. Lower concentrations caused partial kills.

Populations of zooplankton were abundant during pre-treatment sampling of the fertile ponds at Cape Vincent. In contrast with the usual situation, zooplankton declined sharply after Ichthyocide was applied. Coincidentally, however, night temperatures dropped below freezing and may have contributed to the declines. Bottom fauna were not affected.

PHYSIOLOGY

Fate of control agents in fish

Methods for detecting and measuring Thiodan and its metabolites in fish tissues were investigated. Results appeared in Progress Reports for the quarters ending June 30 and September 30, 1964.

The Bratton-Marshall analytical method for sulfa residues was tested as a means of detecting and measuring MS-222 in tissues of rainbow trout. Problems of masking or interfering substances in background were encountered, and measurements of MS-222 in blood and muscle were more valid than those in liver and kidney. At present the method appears to be satisfactory for determining at least the higher levels of MS-222 in tissues.

The efficacy of fish anesthetics

Effectiveness of MS-222, Quinaldine, and methyl pentynol as anesthetics was evaluated for various species and sizes of fish in hard and soft waters of different temperatures and pH. The employment of two temporary assistants during the second half of the year helped to speed the work.

Criteria for desired responses of fish to the drugs were defined. Generally, we sought to determine concentrations of the anesthetics which produce sedation, moderately rapid anesthesia, and complete recovery of fish. Also, effects of sedating concentrations of MS-222 on oxygen consumption by rainbow trout were observed. In autumn, use of MS-222 and Quinaldine in spawning rainbow and brook trout was evaluated at the Wisconsin State hatchery at Osceola and at national fish hatcheries at Genoa, Wisconsin, and Manchester, Iowa.

Hematocrits in fish

An evaluation of the YSI Electronic Hematocrit for fishery use was completed in the third quarter. The final study determined the effects of protein and sodium chloride on readings. A manuscript is in preparation.

MAINTENANCE OF EXPERIMENTAL FISH

In cooperation with the bioassay staff, a study was initiated to determine how long various species and sizes of fish must be held off feed to empty the gut. It appeared that the accepted 3 days was too long for fry- and fingerling-size carnivorous species such as northern pike and walleye. To date, observations have been made on the voiding times of 17 species, and the study is continuing.

GENERAL

The physical plant and facilities of the Southeastern Fish Control Laboratory at Warm Springs, Georgia are nearly completed. A major contract for a wet laboratory-holding house and storage building ended in June, months late. The contract for outside pools, an auxiliary water system, fencing, paving, and grading was completed in December. Research was badly hindered or interrupted by construction activities and water problems.

Some additions and alterations to facilities were made at La Crosse. A shelter for boats and field gear was completed, a water line from the bass pond into the holding house was installed, a large chemical hood was placed in the chemistry laboratory, the meat preparation room was converted to water conditioning, and a second deionizer and associated filters were obtained.

A four-man bioassay team of the Fish-Pesticide Research Laboratory was set up in an office-laboratory in the holding house. The team also uses space in the wet laboratory and pools on the levee for bioassays of pesticides.

Disposal systems for waste waters at La Crosse and Warm Springs were inspected and found satisfactory by Officers of the U.S. Public Health Service.

Dr. Lennon was appointed to the Subcommittee on Vertebrate Pests in the Agricultural Board, National Academy of Sciences - National Research Council. He was also elected Chairman of the Research Advisory Committee to the Wisconsin Conservation Commission for 1964-65.

Mr. Charles Walker was appointed to the faculty of the Biology Department, Viterbo College, La Crosse, in September. He teaches an evening course in ecology.

STAFF

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RESERVOIRS

NATIONAL RESERVOIR RESEARCH PROGRAM

Fayetteville, Arkansas

Robert M. Jenkins, Director

HIGHLIGHTS

Data on 640 large U.S. reservoirs have been assembled and catalogued, including up to 38 parameters describing environmental conditions associated with each impoundment.

Information concerning physico-chemical features of reservoir and inflowing stream waters is partially compiled.

Fish standing crop and/or harvest rate estimates have been located concerning about 250 reservoirs.

An intensive search (by correspondence) for additional unpublished harvest and standing crop data has been instituted.

Arrangements were completed for computer programming and use of an IBM 7040 computer in multivariate analysis of factors influencing reservoir fish production.

A bibliography on reservoir fishery biology has been completed and extraction begun of data pertinent to multivariate analysis from the 1,300 references cited.

Further testing of the two-man dry submarine at Bull Shoals Reservoir in 1964 indicated that its primary utility will be in prolonged underwater observations of fish behavior (spawning, nesting, feeding) in sharply delimited areas.

Specific plans for future research within the Bureau's reservoir program were formulated for two additional reservoir complexes.

Program discussion and field equipment demonstrations were arranged for the Reservoir Committee, Southern Division, American Fisheries Society, the Oklahoma Department of Wildlife Conservation, and California and Missouri fishery biologists.

NATIONAL RESERVOIR DATA COLLECTION

Needed descriptive information has been assembled, recorded, and filed in individual folders on 640 reservoirs over 500 surface acres in area. Parameters catalogued include: 1) location - stream and USGS index number; 2) drainage area in mi.; 3) height of dam; 4) number, size and depth of outlets; 5) number and KW capacity of generators; 6) storage in acre-feet, area in acres and elevation (msl) at flood control, power or irrigation, recreation or conservation, minimum power, and dead storage pool levels; 7) elevation of stream bed at dam; 8) storage ratio as computed by USGS and by using estimated average annual storage volumes; 9) average annual tailwater discharge in cfs. and acre-feet; 10) shoreline length at maximum and average annual pools; 11) shore development at average annual pool; 12) maximum depth and average depth at maximum and average annual pools; 13) reservoir age, uses, owner, and cost; 14) number of public use areas and number with boat dock; 15) fish management facilities present (e.g., fishing piers, brush piles, hatcheries, ladders, etc.); and 16) a map of the reservoir.

Judging from publications and reports gathered in the past 18 months, not more than 100 reservoirs on which some biological information may be available will be added to the study.

Additional independent variables now being recorded on individual reservoirs (where available): Basin vegetation and soil types and length of growing season; inflowing stream sediment concentration, chemical and dissolved solids type and 16 chemical parameters; reservoir conductivity, methyl orange alkalinity, bottom temperature and depth of dissolved oxygen depletion in August; secchi disc transparency, pH, total organic matter, plankton and bottom fauna standing crops.

From reports on hand, it appears that information on harvest rates and/or standing crop of fishes will be available on about 250 reservoirs. However, some of these data may be inadequate for use in multivariate analysis. Further requests for unpublished data will be directed to fishery agencies during 1965. Information sought includes: Sport fish harvest in pounds per acre per year and/or rate of harvest in fish per angler-hour; annual commercial fish harvest in pounds per acre; standing crop of fish as determined by cove sampling or by mark and recapture population estimation. Cove samples will be categorized by method; e.g., with blockoff net, without blockoff net, use of marked fish.

Sport fish harvest will be subdivided into: 1) food chain categories (short, medium and long); 2) predator, prey, rough, forage and harvestable; and 3) closely related groups (e.g., black basses, sunfishes, catfishes).

A great deal of unsummarized age and growth data are presented in the assembled reservoir reports, and an attempt will subsequently be made to organize and analyze this information on the principal species.

SUBMARINE AS A RESEARCH TOOL

Test dives with the two-man submarine in Bull Shoals Reservoir during the summer provided closer definition of its capabilities, limitations, and needed improvements. Capabilities include prolonged submergence for long-term observation, and greater speed and movement without fatigue than is possible with SCUBA gear. Limitations include high maintenance and man-hour requirements, instability of the craft on the surface, uncomfortable

humidity-heat conditions when epilimnion water temperatures exceed 75°F., and restrictions of movement when water clarity is less than optimum.

Improvements scheduled for 1965 include launch and retrieval from a floating barge equipped with hoists, installation of gyroscopic compass and two-way radio, and speedier battery recharging.

GENERAL

Field technical administration included inspection trips to all reservoirs under investigation, a review of work unit progress and projection of future additions and completions based on anticipated personnel and equipment capabilities. Success and difficulties encountered in the field indicate that greater immediate rewards may be forthcoming through increased emphasis on reproduction and early life history and fish behavior studies. Combined efforts of the two investigations teams next year will be divided by project, about as follows: Life history, 40 percent; population dynamics, 30 percent; limnology, 30 percent.

Field equipment innovation demonstrations and program discussions by South Central Reservoir Investigations were arranged for the Reservoir Committee, Southern Division, American Fisheries Society, Oklahoma Department of Wildlife Conservation biologists and law enforcement officers, and California and Missouri conservation agency fishery researchers.

STAFF

Robert M. Jenkins, Fishery Biologist
Judith A. Wilson, Clerk-typist

NORTH CENTRAL RESERVOIR INVESTIGATIONS

Yankton, South Dakota

Norman G. Benson, Director

HIGHLIGHTS

A research station was established at Pierre to work on Oahe and Big Bend Reservoirs. Research will emphasize early life history of yellow perch, northern pike, sauger, and wall-eye.

A 30-day mark-and-recapture population study on Lewis and Clark Lake did not give results with desired levels of confidence except for white crappie.

Knowledge of early life history of the sauger has prompted a request for special water management to experimentally foster a strong year class.

The automatic plankton sampler monitors reservoir plankton populations in Lewis and Clark Lake at low cost. Relationships between outflow plankton and reservoir plankton have been determined.

An analysis of the biological, chemical, and physical data on the entire mainstem reservoir system is in the final stages of preparation.

A new system for measuring bottom fauna biomass describes seasonal changes in a 3,500-acre section of Lewis and Clark Lake.

Adult and young-of-the-year game fish abundance in newly impounded Big Bend Reservoir is high.

FISH POPULATIONS

Population estimate

A mark-and-recovery program commenced during June to determine abundance of major fishes in Lewis and Clark Lake. This information reveals efficiency of sampling gear and the dynamics of reservoir fish populations. Fish were captured by Lake Erie trap nets and frame nets; 17,202 fish were marked by re-

moval of one or more fins. Using the Schumacher-Eschmeyer method, estimates were obtained of adult population size in order of decreasing abundance for carp, river carpsucker, white crappie, smallmouth buffalo, sauger, bigmouth buffalo, and black crappie. Scarcity of freshwater drum and white bass prohibited estimates of their abundance. White crappie and black crappie provided the best estimates. Tag returns were too few to obtain population estimates with desired limits of confidence. Reliable estimates might have been obtained with a four-fold increase in effort.

Population monitoring

Gill and frame net captures in April and May reflected the relative abundance of adult fishes. Compared to previous years, smallmouth buffalo, white crappie, and black crappie appeared to be more numerous and carp and freshwater drum less so. No changes occurred in the year-class structure of fish to account for these differences. Relative abundance of shovelnose sturgeon, shortnose gar, river carpsucker, channel catfish, and sauger was similar to 1963. Differences in catch rates between 1964 and previous years may be attributable to extreme low water this spring which caused fish to move out of shallow water areas and become more vulnerable to capture.

We used a variety of gear to evaluate reproductive success. The 28-foot trawl sampled deep water, the 16-foot trawl sampled shallow water, and the 220 volt DC electroshocker sampled areas with submerged trees and brush. Reproduction in 1964 was good for channel catfish, white crappie, and freshwater drum; average for gizzard shad and white bass; and poor for carp, river carpsucker, smallmouth buffalo, bigmouth buffalo, and sauger.

Big Bend Reservoir (impounded in 1964)

Systematic sampling (weekly trawl and gill netting) of the reservoir began in the late

summer in cooperation with the South Dakota Department of Game, Fish, and Parks. Preliminary analyses of trawl data indicated an abundance of young-of-the-year northern pike, walleye, and sauger. Gill nets in the tailwaters of Oahe or the upper end of Big Bend made excellent catches of walleye and northern pike (table 1).

Sampling of young fish in Oahe

Delineation of spawning and nursery grounds in the Cheyenne River embayment of Oahe Reservoir was begun. This embayment is 15 miles long, has a maximum depth of 90 feet and averages 1.5 miles in width. Fishing gears used were haul seines, trawls, gill nets, and electric shockers. The 100'x8'x0.5' haul seine captured the most numbers and species. Catches were highest in water less than 3 feet in depth; otherwise, there were little differences among the 9 sampling stations (table 2). No northern pike, paddlefish, shovelnose, or pallid sturgeon were captured. Except for young-of-the-year paddlefish these species were also absent from collections of the North Dakota Game and Fish Department and of BCF made in other sections of Oahe Reservoir in 1964. Therefore, the Cheyenne River area may be considered representative of the entire reservoir, and intensive studies will be continued there in 1965.

Table 1:--Average catch per standard gill net set, Oahe tailwaters of Big Bend Reservoir, September-December 1964.

Species	Number
Walleye	26
Northern pike	24
Carp sucker	23
Yellow perch	12
Channel catfish	11
Sauger	9
Goldeye	9
Northern redhorse	7
Hizzard shad	4
Other	3

Table 2:--Average catch per standard seine haul, Cheyenne River, August-September 1964.

Species	Number
Silver chub	127
Yellow perch	101
Emerald shiner	52
Carp sucker	15
Carp	14
Black bullhead	11
White crappie	7
Freshwater drum	7
Channel catfish	6
Goldeye	3
White bass	2
Black crappie	1
Other	1

LIFE HISTORY

Sauger

Observations were made in the tailwaters of Fort Randall Dam in April and May to study the spawning habits of Lewis and Clark Lake sauger. Fish spawned between April 27 and May 7 when water temperatures ranged from 43° to 48°F. Preferred spawning locations were areas containing gravel, rubble, and boulders, and most spawning occurred between sundown and 11:00 PM. After fertilization, eggs became adhesive and probably adhered to the river bottom. Eggs hatched in approximately two weeks and larvae were carried down the Missouri River and into the reservoir.

Extreme fluctuations in water level in Fort Randall tailwaters, caused by power peaking operations, appear to adversely affect survival of sauger eggs and larvae. Comparison of water levels during the spawning period with resultant year-class strength (1956-64) indicated that fluctuation in tailwater levels or discharge variations were significantly correlated with year-class strength. A request has been submitted for reduced water level fluctuation in 1965 to test further the effects of tailwater discharge patterns.

Spawning periods of sauger and walleye in Fort Randall tailwaters overlap, which helps explain the occurrence of walleye-sauger hybrids in Missouri River impoundments. Eggs of both species and of hybrids were cultured and reared to 40 mm to determine distinguishing characters for identifying young fish. Walleye and sauger less than 10 mm long can be differentiated by the shape of the egg sac. Characteristics of the pyloric caeca differentiate fish more than 25 mm long. However, no sample means have been found to identify fish in between, i. e., from 10 to 25 mm. Morphometric and meristic characters of the two species are being studied for this purpose. Characteristics for separating hybrids from both parent species are also being sought.

White crappie

Early life history studies completed on white crappie in Lewis and Clark Lake showed: (1) spawning extended from mid-May to mid-July; (2) spawning was successful only in protected cove areas; (3) young-of-the-year moved from protected areas to the main reservoir when they were about 45 mm long; (4) scales first formed when fish were 16-19 mm long and scalation was complete at 27 mm; and (5) zooplankton was the dominant food. First year growth, calculated from adult fish, indicated very small differences between year classes. Year-class strength was generally low when water levels fluctuated widely during the spawning season; this effect was less pronounced with high water level than with low water levels.

Carp, river carpsucker, smallmouth and bigmouth buffalo

Carp, river carpsucker, smallmouth buffalo, and bigmouth buffalo produced good year classes in 1955, 1956, and 1957 (dam closed in 1955). Reproduction since 1957 has been poor. Some young were produced each year, but except for river carpsucker, few survived to age group I. Analysis of water stages and population structure suggest that rising water level during the spawning periods of these species is necessary for successful

reproduction. Daily fluctuation in water level, because of upstream power peaking operations, may hinder spawning or survival of larvae.

Growth of these fishes was poor compared to that attained in other Missouri River reservoirs or in other areas of the United States. This may be due to relatively high turbidities, high water exchange rate, and associated low food abundance.

Spawning at Oahe and Big Bend reservoirs

To determine the location and time of spawning of major species, Fred June and his team collected weekly samples of ovaries at 6 stations between Oahe and Garrison tailwaters. Maturity indices, derived from a relationship of ova diameter, ovary weights, and fish length, were computed for northern pike, black and white crappie, and will be computed for all major species. The indices will provide a rapid quantitative method for measuring variations in spawning. Studies on black crappie showed that spawning in upper Oahe Reservoir reached a peak about 5 weeks earlier than near the dam; peak spawning at both locations occurred at 18°C. Ancillary information is being gathered on spawning behavior, habitats, size, sex ratio and age composition of spawners, and occurrence of embryos and larvae.

Identification of early life history stages

Weekly haul seine collections have provided complete series of white crappie, carp, carpsucker, channel catfish and freshwater drum for preparation of taxonomic keys of young stages. We have partial collections for 15 additional species.

LIMNOLOGY

Zooplankton

Dr. Bruce Cowell and his team conducted studies on Lewis and Clark Lake to determine zooplankton population densities, species compositions, and factors which influence species abundance. Weekly samples were collected by the automatic plankton sampler located in the powerhouse of Gavins Point Dam, from 10 stations

in the reservoir, and from 5 tributary creeks. Additional studies included: a 24-hour series collected at two-hour intervals; sampling of the mudwater interface with a modified Frolander-Pratt bottom skimmer; and a series of daily comparisons of duplicate samples to measure sampling variability of the high-speed Miller sampler.

Creek samples had large numbers (up to 400 per liter) of organisms in the spring but included rare species that do not contribute significantly to the reservoir standing crop. By late summer creeks had low flows and little or no zooplankton. Overall, creeks do not influence the plankton population dynamics of the reservoir to a significant degree.

Variability of duplicate samples collected with metered Miller samplers was low, generally not exceeding the sampling error associated with counting sub-samples to determine total densities. Coefficients of variability for the latter were less than 10 percent except in periods of extremely low population densities; the sampling season variability mean was only 7.7 percent. Furthermore, an analysis of variance indicated that variability among sampling stations was far greater than that between duplicate tows at the same station. Daily variation at the same station was also considerably greater than the sampling variation.

Analyses of samples collected at the 10 stations in the reservoir indicated that between stations variability was high on any given date, but not when seasonal means were compared. Apparently most zooplankton production in Lewis and Clark Lake occurs in the lower one-third of the reservoir (fig. 1).

Data from the automatic plankton sampler were compared with those obtained with the metered Miller sampler to determine whether the former was representative of the reservoir (fig. 2). Such comparisons indicate that the automatic plankton sampler can be used in Lewis and Clark Lake to monitor changes in total zooplankton densities. Furthermore, operation of this instrument requires considerably less time than the towing of Miller samplers, and weather and equipment mal-

functions do not materially affect collection of samples.

Zooplankton samples have been collected for one year with the automatic plankton sampler. Peak population densities occurred in the winter, under the ice, and in late spring (fig. 3). A gradual increase in population densities occurred during the fall.

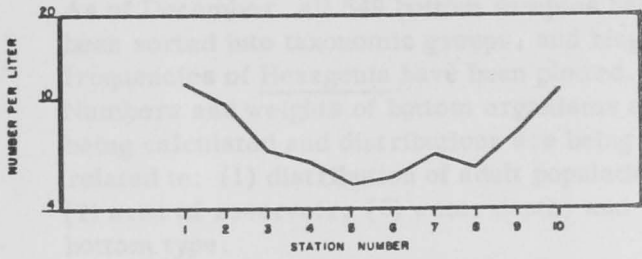


Figure 1:--Mean zooplankton densities at 10 stations in Lewis and Clark Lake (April-October) 1964. Station 1 is near the dam with the other stations at 3-mile intervals upstream. Semi-logarithmic scale.

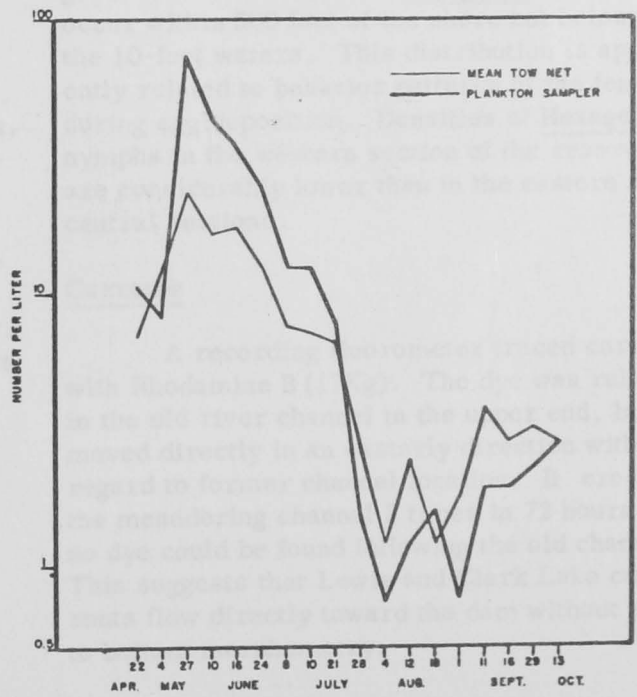


Figure 2:--Comparison of mean tow net (10 stations) and automatic plankton sampler collections of zooplankton, from April to October, 1964. Vertical scale semi-logarithmic.

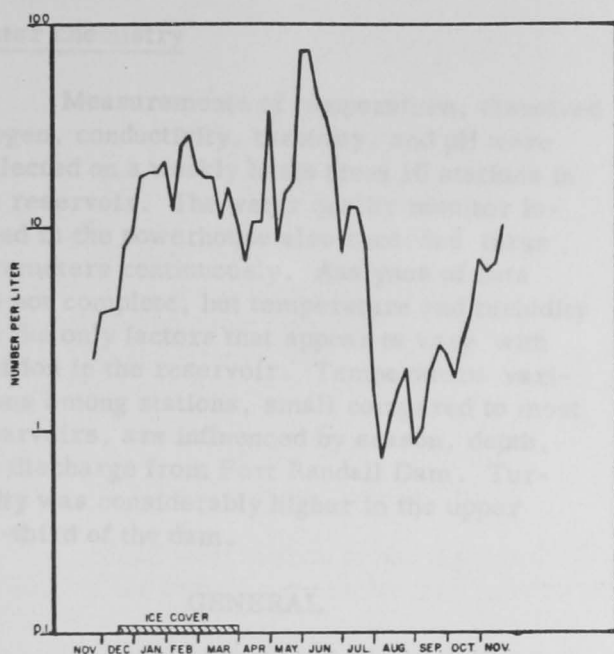


Figure 3:--Zooplankton densities in Lewis and Clark Lake from November 1963 to November 1964, as measured by the automatic plankton sampler.

Comparatively few species predominate during periods of peak populations but many species occur during low periods. There is some indication that species structure and the population density are correlated with water temperature. Additional analyses of species cycles, numbers, and weights are in progress.

Phytoplankton

Phytoplankton densities, similar to zooplankton, showed peak populations in the latter part of May and minimums during late summer. Volumetrically dominant taxonomic groups were diatoms (*Asterionella*, *Synedra*, and *Melosira*) and blue-green algae (*Microcystis* and *Aphanizomenon*). Analyses of phytoplankton data are not complete.

Bottom fauna

A stratified probability sampling was designed to obtain six population estimates (May-October) in a 3,500-acre study area, located in the eastern section of Lewis and Clark Lake. The limnology crew sampled eight

stations on each of six transects distributed in the central and western section of the reservoir at monthly intervals from May to October. Core samples were taken in conjunction with transect samples to determine the relationship between distribution and abundance of organisms and bottom type.

Adult *Hexagenia* were collected at eight stations bordering the reservoir and from two light traps. Examination of species structure of 1,313 specimens collected showed: 92 percent *Hexagenia limbata*; 8 percent *Hexagenia bilineata*; and less than 1 percent *Pentagenia vittigera*. As of December, all 549 bottom samples have been sorted into taxonomic groups, and length frequencies of *Hexagenia* have been plotted. Numbers and weights of bottom organisms are being calculated and distributions are being related to: (1) distribution of adult population; (2) area of reservoir; (3) water depth; and (4) bottom type.

The population of *Hexagenia limbata* has increased over the past three years; *Hexagenia bilineata* is restricted to a small population in the central section of the reservoir. The greatest concentration of *Hexagenia* nymphs occur within 500 feet of the shore but below the 10-foot waters. This distribution is apparently related to behavior patterns of the female during egg deposition. Densities of *Hexagenia* nymphs in the western section of the reservoir are considerably lower than in the eastern and central sections.

Currents

A recording fluorometer traced currents with Rhodamine B (17Kg). The dye was released in the old river channel in the upper end, but it moved directly in an easterly direction without regard to former channel location. It crossed the meandering channel 3 times in 72 hours but no dye could be found following the old channel. This suggests that Lewis and Clark Lake currents flow directly toward the dam without regard to bottom morphology.

Water chemistry

Measurements of temperature, dissolved oxygen, conductivity, turbidity, and pH were collected on a weekly basis from 10 stations in the reservoir. The water quality monitor located in the powerhouse also recorded these parameters continuously. Analyses of data are not complete, but temperature and turbidity are the only factors that appear to vary with position in the reservoir. Temperature variations among stations, small compared to most reservoirs, are influenced by season, depth, and discharge from Fort Randall Dam. Turbidity was considerably higher in the upper one-third of the dam.

GENERAL

Mainstem reservoir survey

Biological, chemical, and physical data on the entire mainstem system have been analyzed in preparation for a comprehensive report. Significant findings were: (1) density currents, as measured by sum of water density, total dissolved solids, and suspended solids, were detected only in the upper end of Fort Peck and were of a temporary nature; (2) thermal stratification, persistent in Fort Peck, was commonly disrupted in other reservoirs due to wind and currents; (3) in Garrison and Fort Peck, most incoming suspended sediment was deposited within 25 miles of entry; (4) it may be possible to reduce turbidities caused by wind on several reservoirs by altering water levels; (5) total dissolved solids of all large reservoirs increased toward the dam but Garrison showed extremely variable chemical patterns; (6) average summer surface water temperatures are 10° F. less in Fort Peck than in Lewis and Clark, with average temperatures of intermediate reservoirs increasing with decreasing latitude; (7) phytoplankton abundance as remained constant in the upper part of the system but has increased considerably since 1953 at Yankton; (8) gill net catch data indicates fish abundance was highest in Garrison and lowest in Lewis and Clark; (9) in all reservoirs, predator species such as walleye, sauger, and northern pike reproduced sporadi-

cally, but their relative abundance was increasing; (10) short food chain species (buffalo, carpsucker, carp) also produced year classes sporadically but are not increasing; (11) the emerald shiner is developing into the dominant forage species in Big Bend, Oahe and Garrison; gizzard shad are important in Francis Case and Lewis and Clark; and (12) goldeye and drum, both species with semi-buoyant or pelagic eggs, are producing year classes more regularly than species with more specific spawning habitat requirements.

Submerged trees

Plankton, bottom organisms, and periphyton have been collected in Lewis and Clark Lake and in Lake Francis Case. Total floral growth is being measured by cell counts and analyzing acetone-methyl alcohol pigment extracts with a Beckman DK ratio recording spectrophotometer. Concentrations of chlorophylls a, b, c, and 2 carotenoids are being determined.

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SOUTH CENTRAL RESERVOIR INVESTIGATIONS

Fayetteville, Arkansas

Thomas O. Duncan, Director

HIGHLIGHTS

Simultaneous comparative study of a twelve-year-old impoundment (Bull Shoals Reservoir) with one impounded in January, 1964 (Beaver Reservoir), was begun. The new reservoir reached only 15 percent of power storage volume and one-fourth of power pool surface area during the year, complicating sampling procedures and schedules.

Food habit studies of six centrarchids taken in the littoral zone of the mid reaches of Bull Shoals Reservoir indicate that immature aquatic insects form the major component in the diet of all sunfish and bass under four inches in total length.

Efforts to identify fish larvae collected in open water and inflowing streams in both reservoirs revealed a paucity of information available on the early life history stages of many species.

Midwater trawling demonstrated vertical layering of various size groups of fishes, primarily gizzard and threadfin shad. Midwater trawl catches at night were greater than those taken during daylight hours and more effective on young than adult fishes.

Cove rotenone samples in Beaver Reservoir indicated distinct population changes between 1963 and 1964.

Beaver Reservoir had a large volume of oxygen-depleted water (1.6 to 0.8 ppm) during its first fall turnover.

Fluorescent dye introduced in the headwater of Bull Shoals Reservoir in May indicated inflowing water moved 55 miles down reservoir in 30 days, with major flow between the 20 and 80-foot depths.

LIFE HISTORY

Food habit study

A study of the seasonal food habits of longear sunfish, green sunfish, bluegill sunfish, largemouth bass, spotted bass, and smallmouth bass - major inhabitants of the littoral area of Bull Shoals Reservoir - neared completion. Samples were taken with an electroshocker at night from April through November.

Fish captured were grouped into four size categories: 0-1.9, 2.0-3.9, 4.0-7.9, and 8.0 plus inches, total length.

Stomachs were pooled by species within length groups for analysis. A total of 1,459 stomachs were analyzed from the ten collections. Immature aquatic insects, predominately Tendipedidae and Ephemeroptera, were a major food for sunfish and bass under four inches. Microcrustaceans were important in the diet of smaller sunfish and bass. The three basses above 8 inches fed almost entirely on fish. The three species of sunfish above 4 inches showed distinct food preferences. Green sunfish fed almost entirely on crayfish throughout the sampling period. Longear sunfish relied primarily on terrestrial insects and secondarily on immature aquatic insects during the mid-summer. Bluegills consumed terrestrial insects, aquatic insects, filamentous algae, and organic detritus in approximately equal quantities.

There were three periods when certain food items were available in such abundance that almost all fishes fed upon them. The first of these periods occurred in early May, at the onset of bass spawning, when fish eggs were a major food component of the sunfishes. The second period occurred in mid-August when a large population of midge larvae and mayfly naiads became available. Midge larvae 3 mm. in length were taken in large quantities

by all fishes under four inches and by the larger bluegills and longear sunfish. In mid-August large schools of young-of-the-year threadfin shad appeared throughout the reservoir. They provided the bulk of the food consumed by bass throughout late summer and fall.

Rainbow trout stomachs were collected over a six month period, April through September, at a public boat dock near the dam on Bull Shoals Reservoir. A total of 49 stomachs have been examined from the first four months sampling. Shad 30-140 mm. made up the bulk of the diet in April. Trout collected in May had been scavengers at the boat docks. Excluding fish entrails and cigarette butts, terrestrial insects were the main food. In June 72 percent by volume of the stomach contents consisted of planktonic organisms primarily *Daphnia galeata* and *Chaoborus*. Shad and *Chaoborus* were the principal food items in July.

Early life history

Collections of young fish obtained with towed and set meter nets in both reservoirs have been sorted for early life history studies. Positive identification of all larval and juvenile stages is presently uncertain. Additional collections will be necessary to secure complete size series before many species can be positively identified.

Underwater observations

SCUBA divers observed fish behavior in various habitats and at different times of the day and year in Bull Shoals Reservoir. Feeding, nest attendance and resting have been observed for several species. Diving at night revealed many fish lying at rest on the lake bottom in waters 5 to 20 feet deep. The "sleeping" basses and sunfishes could be approached very closely with a hand light before they moved away. Equilibrium appeared to be disturbed as they swam. Further observations of this phenomenon are planned in an attempt to answer questions raised by the success of angling at night for bass and night mid-water trawl catches of centrarchids.

POPULATION DYNAMICS

Creel census

A creel census design based on a stratified sampling pattern was prepared for Beaver Reservoir. The design employs stratification by seasons, week days and weekend days, periods of daylight hours, and areas of the lake. Angler usage estimates are derived from counts made from boats and aerial flights. In addition, automatic traffic counters installed at all access points provide an estimate of the total visitor attendance.

The census began in June as a cooperative effort of the Arkansas Game and Fish Commission, the U.S. Army Corps of Engineers, and the Bureau of Sport Fisheries and Wildlife. The reservoir only filled to 7,000 surface acres (15 percent of power pool storage volume) this year, and extensive forests of partially submerged trees hampered strict compliance with the sampling design.

Midwater trawl development

Fish population sampling in clear, steep-sided, rough-bottomed Bull Shoals Reservoir with conventional equipment has proven inefficient.

Echosounding revealed that the midwater zone is occupied by great numbers of fish. Only the midwater trawl affords an adequate method for sampling these waters at widely separated points within a short time.

A 22-foot flatbottom boat was rigged for trawling small midwater nets. Test fishing and modification of trawling gear occupied much of the time allocated for fish population sampling during the year. With the use of an echosounder as a fishfinder, the trawls were tested in four White River reservoirs. Trawling at night produced much better catches than during the day. The trawl could be fished at any desired depth down to 60 feet and used to sample many parts of a reservoir in a short time.

Fifteen species of fish have been captured in the midwater trawl, primarily young fish. The maximum towing speed attained was 4.5 miles per hour, believed to be too slow for efficiently capturing adult fish.

In all lakes except Beaver Reservoir, gizzard and threadfin shad comprised the major portion of the catch. Two distinct layers of fish were usually visible in the echograms. Night tows through these layers revealed numerous young shad near the surface (0-15 feet) and adults below (15-30 feet). Other species captured usually occurred in the lower layer.

Four 20-foot deep, unweighted gill nets fished the midwater zone to provide comparisons with midwater trawl catches. Catches by both methods were similar for young-of-the-year fishes. However, gill nets took many adult fishes rarely captured by the trawl.

Catches in Beaver Reservoir were dominated by young-of-the-year gizzard shad in the early summer but by late summer black bullhead, white bass and black crappie predominated.

Encouraged by the results from midwater trawling, a larger boat powered with a 55 hp diesel engine was purchased and outfitted in December. Higher trawling speeds should provide bigger and more representative samples of the adult population.

Beaver Reservoir population estimates

Two fish population samples were taken with rotenone in Beaver Reservoir in September employing the same procedures as in 1963. Fish population estimates of each sampling area were calculated for all species that provided recaptures (table 1).

Table 1:--Comparison of Beaver Reservoir fish population samples in 1963 and 1964.

Species	Age Group	Fish per acre		Pounds per acre	
		1963	1964	1963	1964
Largemouth bass	O*	115	617	63	76
	A*	22		6	
Spotted bass	O		14		1
	A	24		10	
Black crappie	O	14,557	9	287	1
White crappie	O	701		15	
Walleye	O	29		2	
White bass	O		150		4
Channel catfish	A	14	3	1	3
Flathead catfish	A		1		4
Black bullhead	O		635		25
Longear sunfish	A	515	624	21	41
Bluegill	A	70	102	5	15
Green sunfish	A		92		3
Gizzard shad	O	5,994	2,227	123	285
	A	5	183	2	86
Golden redhorse	A	10	1	3	1
Black redhorse	A	3		2	
Redhorse Sp.?	A	5			
Golden shiner	A	3	15		1
Log perch	A		36		
Carp	O	1,156	1,049	127	234
	A		6		12
TOTALS		23,223	5,764	667	792

*O = Age group 0

*A = Age group I+

At the time of the 1963 sample the lake contained a dead storage pool of 400 acres. In 1964, it had expanded to 7,000 acres.

Largemouth bass numbers increased sharply in 1964; estimates were 617 fish per acre, compared to 115 in 1963. The standing crop also increased, but growth was slower and average size smaller. Samples indicated an exceptionally large population of black crappie young-of-the-year in 1963 but few were taken in 1964. However, midwater trawling produced large catches of both the 1963 and 1964 year classes.

High reproduction of white crappie was evident from the 1963 samples, but none was taken in 1964. The reverse was true of white bass and bullheads. Sunfish populations all increased, both in numbers and pounds per acre, this year.

The numbers of young gizzard shad recovered in 1964 was less than half that in 1963 but weight more than doubled. Much faster growth was evident for young shad in 1964. Adult shad, represented almost exclusively by the 1963 year-class, accounted for the large weight increase.

LIMNOLOGY

Periodic monitoring of various limnological parameters at 14 stations continued through 1964 on Bull Shoals Reservoir and began in June at 8 stations on Beaver Reservoir.

Temperature

Bull Shoals Reservoir:--The water mass of Bull Shoals cools very slowly in the fall, reaching minimum temperature conditions (38.3-45.0°F.) in early February. Temperatures below 40°F. were restricted to the main channel 50 miles or more upstream from the dam. Spring warming was underway by early March, and by late March, surface waters reached 50°F.; 60°F. by late April; 70°F. between mid and late May and 80°F. in June. Stratification began in May.

Water level fluctuation and stratification patterns were similar in 1963 and 1964. In early summer, with the metalimnion positioned between the 20-25 to 60-70 foot depth levels, the epilimnion, metalimnion and hypolimnion each constituted about one-third of the total reservoir volume. Progressive sinking of the metalimnion occurred with the epilimnion about doubling in volume and the hypolimnion and the metalimnion each contracting about one-third by mid-October. Stratification did not completely disappear until about January 1 in both years. Temperatures did not decline below 44°F. in the profundal zone.

Beaver Reservoir:--During 1964 Beaver Reservoir averaged about 7,000 acres in surface area (25 percent of ultimate power pool area of 28,000 acres and 15 percent of its volume). Maximum depth reached was 116 feet. Stratification was acute by late June, with the thermocline positioned between 10 and 15 feet. By late August the thermocline was depressed to 15 to 20 feet, and by mid-October to 30 feet. The overturn was completed in November. Average temperatures were slightly higher in Beaver than in Bull Shoals.

Dissolved oxygen

Bull Shoals Reservoir:--With the advent of stratification, oxygen depletion below the thermocline was accelerated in the upper end midreaches but not in the lower reservoir. Orthograde conditions are gradually replaced by negative heterograde conditions as summer progresses into fall in the lower reservoir. In this area the oxygen content is not reduced below 4.0 ppm in the metalimnion and some oxygen (1-2 ppm) remains until the overturn. Clinograde conditions develop in the arms and upper reservoir shortly after the onset of sharply defined stratification in June. The mid-reservoir main stem displays an intergrade between these two extremes.

Beaver Reservoir:--Oxygen was depleted below the thermocline by June, except for a 1.0 to 2.0 ppm cell located between the 35 and 75-foot depth level in the vicinity of the dam. By mid-October surface values were down to the

4.0 - 4.8 ppm range at the dam. Upstream for 20 river miles, oxygen values ranged from 2.9 to 3.8 ppm. In the next six river miles upstream, surface values dropped to 1.6 to 0.8 ppm (6 to 17 percent saturation). Upstream, oxygen content gradually increased to 100 percent saturation at the inflowing White River. In mid-December, the area of lowest oxygen content (1.2 to 1.8 ppm) had moved down reservoir to six miles above the dam and graded to a high at the dam of 2.0 - 2.7 ppm (18-24 percent saturation).

Conductivity

Bull Shoals Reservoir:-- Specific conductance values reflect considerable complexity within a recognizable seasonal pattern. During stratification there is an abrupt increase in the concentration of ions within the thermocline. Epilimnetic waters during this period displayed conductance values ranging from 200 to 270 micromhos (at 25°C.), with values above 245 micromhos recorded in upper portions of the reservoir. Below the thermocline in the latter zones and in the mid-reservoir main stem, values gradually built up to the range of 300 to 340 micromhos on the bottom prior to the fall turnover.

The years 1963 and 1964 are best described as very similar drought years with attendant low flows and water levels. Under such conditions it would be expected that conductivity would increase and observations in the two years indicate that it did.

Beaver Reservoir:--Conductivity values during 1964 displayed a wide range in values (130-280 micromhos). Highest values were associated with areas of maximum deposition in the upper third of the reservoir and near the bottom.

Dissolved organic matter

Dissolved organic matter (Maciolek method) was fairly uniform throughout Bull Shoals Reservoir, both vertically and horizontally, in April, but exhibited much vertical and between station variation in June and August.

Values in April ranged from 4.0 to 6.8 mg/l compared to 5.2 to 10.7 mg/l in June and August. In November, values dropped to 5.1 to 6.8 mg/l. Beaver Reservoir also demonstrated wide fluctuations with depth during the summer, ranging from 6.7 and 10.5 mg/l. Values decreased to 7.1 to 8.0 mg/l in December. Dissolved organic matter levels were similar in the new and the 12-year-old reservoir during the summer, but were higher in the new reservoir in early winter.

Currents

The flow pattern of water entering Bull Shoals Reservoir was studied through the spring and summer months employing the fluorometer-rhodamine B dye technique. The results of these tests indicated that the dye-tagged inflowing water moved 55 miles down the reservoir in 30 days. Vertical differences in dye concentrations indicated major water movement under the thermocline between depths of 20 and 80 feet. High concentrations detected in some areas indicated settling out below 80 feet and pocketing in side eddies.

Other water chemistry

Seasonal determination of total phosphorus, nitrate, alkalinity, hardness and silica at four stations on Bull Shoals Reservoir at 40-foot-depth intervals has been completed. A similar effort began in June at Beaver Reservoir. In addition, two series of analyses, one summer and one winter, from these same stations on both reservoirs have been made by the Taft Center, USPHS, Cincinnati, for 24 elements. The Ohio Agricultural Experiment Station has processed four seasonal water sample series for 14 elements from these same stations and depths on Bull Shoals Reservoir, and three series from Beaver Reservoir.

GENERAL

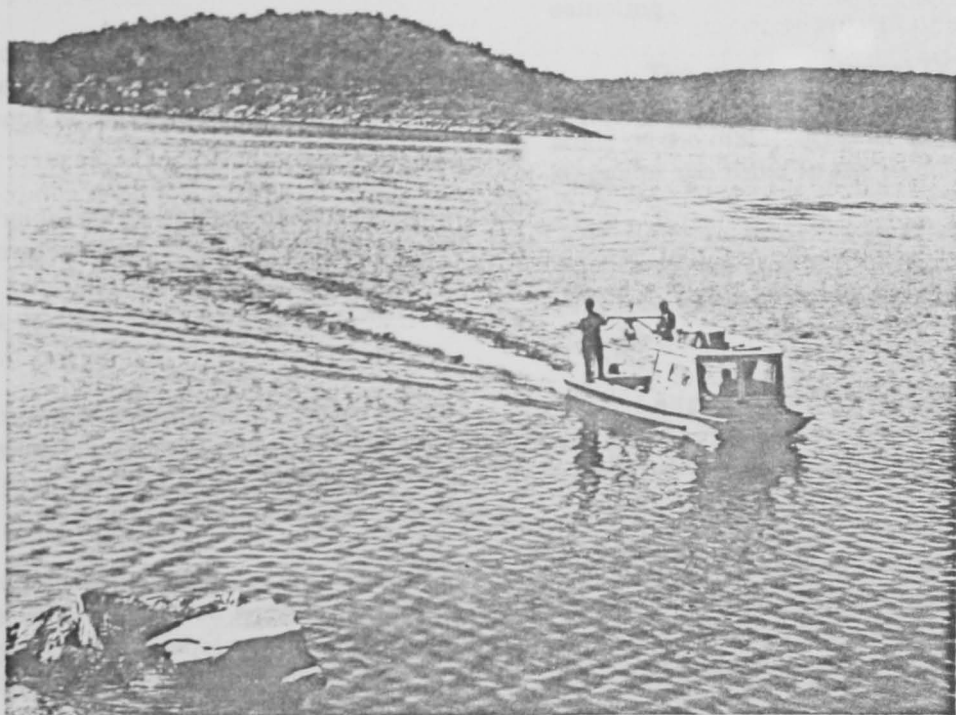
Plans for a floating barge, designed to function as a Beaver Reservoir field operating base and laboratory, were completed in October. The barge will be 20x60 feet, with a prefabricated building 12x24 feet placed on one end. Cranes will be located on the bow for lifting the two-man submarine and small boats to the deck.

Final reports on the Beaver Reservoir preimpoundment research were submitted by the University of Arkansas. A summary of the results of all six reports is in preparation. Several are expected to be published in various journals during 1965. A new 2-year contract with the University of Arkansas was negotiated during the year. The research projects include: (1) early life history of the basses; (2) continued research on bass parasites; (3) sedimentation and water chemistry research; and (4) taxonomy of the aquatic insect fauna.

A contract with Auburn University for research on aquatic insect ecology was negotiated in 1964. Summer field work was terminated on September 15, 1964 and the remainder of the year was devoted to slide preparation of midges for identification and analysis of data. Tentative 1965 plans call for the use of multiple-plate samplers and submerged funnel traps in attempts to describe quantitatively some aspects of bottom fauna production.

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Midwater trawling in Tenkiller Reservoir, Illinois River, Oklahoma. Taken during demonstration in October, 1964.

Note: In above photograph, hydrofoils (lifters) can be seen on surface near the end of white prop wash.

MARINE

SANDY HOOK MARINE LABORATORY

Highlands, New Jersey

L. A. Walford, Director

HIGHLIGHTS

In behavioral experiments bluefish were conditioned to sound stimuli showing that bluefish do hear and that physiological recording is an effective technique.

Systematic observations of bluefish feeding have shown that bluefish locate prey by sight but that a feeding stimulus is produced by olfactory sense.

Results from tagging 8,178 bluefish indicate the presence of major groups of bluefish along the Atlantic Coast, each apparently divided into sub-groups.

A completed study of parasites of bluefish gave evidence that there are at least three major groups of bluefish along the Atlantic Coast.

In underwater studies divers have observed the spawning rituals of cunner and the feeding of bluefish both peacefully with round herring in daytime and savagely on them at night.

Attempts to culture both bluefish and fluke eggs have been successful through hatching to five days of larval life and have produced complete study collections of earliest life stages.

Studies of the acute toxicity of nine insecticides were completed showing variation in toxicity for snails, shrimp and many species of fish with salinity and temperature.

Toxicity studies showed that insecticides affect electrolyte metabolism of fishes.

In Navy-supported studies, 365 sharks, principally blues and sandbars, were tagged and four tags were returned.

Experimental fishing showed that sharks were in moderate abundance in the New York bight area in 1964 with a catch rate of 2 per 100 hooks.

Two dinoflagellates were identified as the cause of a fish kill in Barnegat Bay, New Jersey.

Toxicity of insecticide Apholate was shown to act by blockage of purine and pyrimidine formation thus preventing DNA synthesis.

A contract study showed that it would be feasible to employ small roaming units to obtain sport fish statistics through systematic sampling.

The research vessel DOLPHIN was employed in a large-scale interstate cooperative study of the distribution of eggs and early life stages of the fluke in the middle Atlantic.

The monthly ocean temperature program showed spring and fall temperatures warmer in 1964 than in 1963, but summer temperatures equivalent.

Aerial spotting showed that Atlantic mackerel migrate through the middle Atlantic to Cape Cod in a narrow temperature band between 9 and 12°C.

The sea tank for behavior studies on large schooling fish was essentially completed including controls for sound, temperature, light and other ambient conditions.

The research vessel DOLPHIN was put into operation late in the year and shows promise of giving excellent service for coastal research.

Plans for the new Narragansett laboratory were essentially completed and reviewed with construction scheduled to begin next spring.

A new 80-tank aquarium facility was put into operation to study the effects of water quality on aquarium fishes. This study is being sponsored by the new National Fisheries Center and Aquarium.

The student apprentice program matured with 40 high caliber laboratory and field assistants assisting in 10 projects throughout the summer.

AERIAL TEMPERATURE SURVEY

Middle Atlantic

Stone and Azarovitz continued and expanded aerial sea surface temperature surveys in 1964 with the cooperation of the U.S. Coast Guard. Surface temperature charts prepared monthly now include the Atlantic Shelf waters from Cape Cod to Cape Henlopen.

Southern New England

This survey was initiated in cooperation with the U.S. Navy Underwater Ordnance Station, Newport, Rhode Island and included the shelf waters between Cape Cod and Montauk Point. Detailed coverage was given to the mouth of Narragansett Bay and the Sakonnet River.

Special surveys

Several surveys of the Florida east coast shelf waters were made in cooperation with the U.S. Coast Guard and Woods Hole Oceanographic Institution. Surface temperatures were collected and drift bottles and sea bed drifters released in an attempt to deduce the effect of water transport and temperature on the distribution of eggs and larvae of bluefish spawned along the Florida east coast.

A special flight was made during December in cooperation with Woods Hole Oceanographic Institution. This aerial sea surface survey covered the Atlantic Shelf from Nova Scotia to Key West. It is hoped that this flight will demonstrate the feasibility of coordinated temperature surveys by marine laboratories along the coast.

An interagency thermal oceanographic study (ITOS) was carried out during June 22-26, 1964. Personnel of the Sandy Hook Marine Laboratory, Coast and Geodetic Survey, U.S. Weather Bureau and the U.S. Coast Guard participated in the study to explore variables affecting the accuracy of infrared techniques for measuring absolute temperature of sea surface. Evaluation of data for June 25th and 26th resulted in radiometric surface temperatures essentially one degree celsius cooler than a contact thermistor at 6 inches below the surface. However, strip chart traces for the two instruments were almost identical indicating close correlation between radiometric and conventional surface temperature measuring devices under certain meteorological conditions.

BIOLOGY OF MICROORGANISMS

Isolation and culture

Prager and Mahoney are maintaining a monthly check list of all phytoplankton species present in significant numbers in lower New York harbor. Samples from 20 stations ranging from estuarine to open sea give a representative picture of the phytoplankton population throughout the year. This information is augmented by notes on the amount of organic detritus at each station.

The "Clay Pit Creek isolate" has been identified as Gymnodinium mirabile, and is growing well in chemically defined medium. A small dinoflagellate, tentatively identified as Hemidinium has survived single cell isolation procedures, and several genetically pure clone cultures are available for nutritional and physiological studies. A positive identification of this species is expected soon. Attempts to establish Polykrikos kofoidi and P. barnegatensis in axenic culture failed, but these species are being carried in a mixed culture of algae and bacteria. No member of this genus has ever been cultured successfully in the absence of bacteria and they are believed to be phagotrophic feeders. This does not preclude their successful culture however, and attempts will be continued because the bloom from which these species were taken caused considerable fish mortality. (see Fish Kill Studies).

Physiology and nutrition

Empirical evidence from nutritional supplementation experiments on Platymonas subcordiformis indicate that Apholate, an insect chemosterilant, acts by inhibiting purine and pyrimidine nucleotide synthesis. This would explain the lowered DNA concentrations found by others in eggs from fish exposed to Apholate. Prager and Mahoney found that the toxic effects of Apholate could be overcome partially by additions of mixed purines, thymine, thymidine, guanine, guanosine, xanthosine, uridine, adenine and were completely reversed by adenosine and triphosphopyridine nucleotide. This fits earlier experimental data which showed that vitamin B₁₂, folic acid, and para aminobenzoic acid partially overcome Apholate effects, since these vitamins are needed to form the purine ring in normal metabolism. Thus, the chemosterilant activity of this ethylene imine appears to be based upon its inhibition of DNA synthesis through blockage of purine and pyrimidine nucleotide formation. These data are the basis of a manuscript to be published in the coming year.

Hemidinium sp. utilizes unusually high concentrations of vitamin B₁₂ and both inorganic and organic phosphates. Organic phosphate sources support densest populations, which is of ecological note. Its experimental salinity toleration is that of a euryhaline, brackish water dinoflagellate. The lower limit of vitamin B₁₂ utilization however, is 0.001 microgram atoms per liter which, from the literature, seems to be a universal level among marine microorganisms. Present media developed for Hemidinium sp. yield concentrations of 500,000 cells/ml and it is quite suitable for vitamin B₁₂ assay of sea water. Nutritional studies on this species are the subject of Mr. Mahoney's M.S. dissertation at New York University, and will be submitted for publication during 1965.

Experimental variations of day length have a profound effect upon algal productivity. Optimal photoperiod limits the mean cell volume and increases the percentage of biovolume represented by chlorophyll "a" in Platymonas subcordiformis cultures, relative to constant

light incubation and non-optimal photoperiod. During optimal photoperiod, smaller mean cell volume increases the population's surface area/biovolume ratio, allowing each cell to utilize relatively more chlorophyll "a" than larger cells and thereby absorb light more efficiently. This photoperiod effect, also observed in Hemidinium sp. and Porphyridium cruentum, demonstrates a weakness in the use of chlorophyll "a" as an index of marine productivity, since that technique assumes a constant ratio of chlorophyll "a" to biovolume (or at least, amount of plant food material present). The photoperiod effect on cell volume also implies that morphometrics of laboratory-grown algal cultures must be taken as valid only when cultures are incubated under diurnal cyclic conditions.

Fish kill studies

Barnegat Bay, N. J. was choked with a dense reddish-brown dinoflagellate bloom of Polykrikos kofoidi and P. barnegatensis during most of August. This resulted in the death of many Menidia, Fundulus, sticklebacks, silver perch, toadfish, eels, and crabs. A full report of this fish kill is being prepared by Prager in cooperation with Paul Hamer, Principal Biologist of the New Jersey Department of Fish and Game.

ANALYSIS OF ENVIRONMENT STUDY OF DISTRIBUTION

Wicklund and assistants have completed assembling, from published and unpublished sources, past records of sea temperatures for the Atlantic Continental Shelf and are now processing the data for mapping. The final drawings will be in the form of three dimensional block charts representing monthly average temperature masses from surface to bottom. Data, of which there are more than 280,000 items, include bathythermograph records and standard hydrograph stations.

NATURAL HISTORY OF BLUEFISH

Early life history

In July Deuel and Clark collected ripe bluefish from pound nets off southern Long

Island. They fertilized the eggs artificially, kept them alive through incubation and reared the larvae for nine days after hatching. They preserved eggs and larvae at various stages of development for later study. Representative specimens were sent to Mrs. A. J. Mansueti of the Chesapeake Biological Laboratory, who will make illustrations to be included in the atlas of eggs and larvae on which she is working.

Observation in natural habitat

During the summer Wicklund and assistants resumed studies begun in 1963, on Shrewsbury Rocks, a natural fishing reef. Using a protective cage as a base of operations, they began a long-range project to chart physiography of the bottom and the distribution of animals and plants in relation to observed features of habitats. At the same time they observed and described behavior of fishes through the 24-hour cycle, including territoriality and spawning ritual of cunner; the young of bluefish feeding peaceably with young mackerel and adult round herring at night; adult bluefish attacking round herring at night; trails of luminescence revealing the attacking patterns of bluefish in total darkness; striking differences in the growth of mussels and algae above and below the thermocline; and movements of cold water masses at the bottom.

Age and growth

Wicklund, Deuel and assistants extended the sampling of bluefish catches to include New York, New Jersey, North Carolina and Florida. They have collected data on lengths and stages of maturity from 7,000 specimens and also scales from 7,000. Tentative conclusions from preliminary analysis of this material are consistent with tag returns, in indicating that bluefish schools keep their identity for several weeks or months. They are available to inshore anglers for only brief periods such as one to three weeks, then vanish for an interval to return later for another brief sojourn, sometimes in the same area but sometimes several miles away. Older fish live farther offshore than younger ones. Evidently the population structure of bluefish is exceedingly complex.

Tracing migrations

In June 1963 we began trials of various types of tags for a bluefish migration study. From laboratory trials with 7 tag types and field trials with 3 types, we chose the dorsal loop spaghetti tag as best. During 1964 Deuel and assistants conducted full scale bluefish tagging with the loop tag. Assistance in tagging was provided by Hassler of North Carolina (under contract), and Smitherman at Louisiana (Cooperative Unit) and a limited number of volunteer sportsmen in Florida, New York and New Jersey.

By the year's end 8,178 bluefish had been tagged in various coastal areas and 455 tags had been returned as shown below:

<u>Area</u>	<u>Dates of Tagging</u>	<u>Number Tagged</u>	<u>Number Returned</u>
New York	June-Sept.	1547	41
New Jersey	June-Aug.	3169	177
Virginia	May	266	17
No. Carolina	June-Sept.	1496	140
So. Carolina	May	19	1
Florida	Feb.-Apr.;		
	Oct.-Dec.	1651	79
Louisiana	November	30	0
		8,178	455

Bluefish for tagging were taken mainly by gill net and pound net. The operation was greatly improved in mid year when we obtained our own gill net, power launch and professional fisherman.

The tag return data for 1964 indicate different migration patterns for two groups of Atlantic coast bluefish. In the north, blues appear to range between Virginia and the New York-New Jersey area, and in the south from Florida to North Carolina. The bulk of returns has come from the tagging vicinity within a few weeks after tagging. The limited number of long distance returns showed the following pattern: In many instances long distance returns came in sets of two or three from fish from the same tagging area indicating that "schools" of bluefish remain intact for at least several months.

Three bluefish tagged in May in Virginia were recaptured near Sandy Hook in July. Returns

from tagging in New Jersey and New York ranged as far north as Massachusetts in summer and as far south as Virginia in the fall. Returns from spring tagging in southern Florida showed a movement northward as far as Jacksonville. One out of 30 fish tagged in South Carolina in May was recaptured in North Carolina in July. From tagging in North Carolina in August and September two fish were returned from Florida in October.

Anatomical features as indicators of races

This project has been relatively inactive this year pending development of methods to speed up the task of measuring body proportions. Preliminary experiments using photography for anatomical studies indicate the need of extensive work to solve the problem of distortion of vertical and horizontal dimensions.

Contract with North Carolina

In cooperation with the laboratory's main tagging program, Hassler and assistants tagged 1,520 small bluefish between June 4th and October 1st in the area between Oregon Inlet to Atlantic Beach. Most were taken by haul seine. The Sandy Hook type loop tags were used.

Returns to date number 161; 145 from commercial fishermen and 16 from sportsmen. Most were returned from the inside waters and within three weeks after tagging.

Other associated observations were: sexually advanced bluefish were observed offshore in mid May; no spring migration north along the Outer Banks was observed; bluefish were found in stomachs of swordfish taken 50 miles offshore in the winter.

BIOLOGICAL ASSAY

Development of techniques

Chlorophyll "a" is widely used as a quantitative measure of marine primary productivity. Our experience indicates that this method is qualitative at best, technically diffi-

cult to perform, inordinately expensive, unsuitable for shipboard use, and its basic assumption that the amount of chlorophyll "a" in a given quantity of sea water is directly proportional to the primary production, is wrong. The chlorophyll "a" content of eight species of cultured algae differs from their mean cell volume by factors of 50 and above. Also, in a single species grown under varying day lengths, chlorophyll "a" content varies independently of the volume of cells produced.

The chlorophyll "a" to biovolume ratio is of value in judging physiological condition of an algal population, in evaluating respiratory potential of a population, and pigment analyses are needed in algal systematics. Prager and Lekach have reduced the pigment extraction time required from 12-24 hours to 1-3 hours using improved techniques.

Vitamin B₁₂ assay trials using Hemidinium sp. await final improvements in the medium.

Prager is testing a product to attract game fish. This is a surface drifting cloud of colloidal clay particles which is expected to serve as a shelter for pelagic fishes comparable to a raft. Preliminary 60-day laboratory tests of various clay concentrations on pure cultures of algae, killifish, grass shrimp, hermit crabs, and adult quahogs showed no toxicity or debilitating effects. Two preliminary field trials have been made.

Biochemistry of sea water

Data on dominant and subdominant algal species, dissolved O₂, salinity, temperature, chlorophyll "a" organic phosphate, total vitamins, total trace metals, B₁₂, iron, and organic nutrients are taken from surface samples at 20 stations in lower New York harbor monthly. Echo soundings give some rough indication of abundance of fishes in the water. These data will be evaluated at the end of a year's survey next April.

NATURAL HISTORY OF SHARKS

Systematics

Casey continued to strengthen description of species of large sharks by making detailed measurements on samples of 122 specimens representing the following species: white, mako, hammerhead, sandbar, dusky, angel, thresher, bull, blue and sand sharks.

The shape and relative position of fins, eyes, and other morphometric features are useful characteristics for identifying and comparing differences between species of sharks. However, comparisons of proportional measurements which will readily separate adults of similar species are of little value in comparing different sizes and ages since body proportions change with age.

We now have sufficient data to show morphometric changes with growth for some of the above species. These data will be included in a forthcoming publication.

Tracing migrations

Casey, with assistance from summer assistants and volunteer sportsmen tagged and released 365 sharks. These included 111 sandbar, 115 blue, 27 sand, 23 dusky, 5 bull, 59 dogfish, and 25 miscellaneous sharks, skates, and rays. Four sharks were subsequently recaptured. Two blue sharks tagged off eastern Long Island were recaptured in the same area after 4 and 51 days respectively. A smooth dogfish tagged off northern New Jersey was recaptured 150 miles off Fishers Island, N. Y. after 31 days. A sandbar shark tagged on the eastern side of Delaware Bay was recaptured in the Bay after 47 days and 25 miles from where it had been tagged.

A major problem which must be resolved before an extensive volunteer shark tagging program can be undertaken centers on developing a suitable dart tag. Dart tags which have been successfully used for many years on tunas, marlins, and sailfish are not satisfactory for sharks. We have put considerable effort, there-

fore, into designing and testing tags which sportsmen can easily insert into large specimens without bringing them on board. Most of these have proved unsatisfactory.

We are currently testing a modified version of a dart tag designed by Frank Mather of the Woods Hole Oceanographic Institution. These tags have been placed on aquarium sharks in the U.S. and South Africa. Preliminary results are encouraging and this modified "M" tag will be sent to taggers in the spring. This year the American Institute of Biological Science began an international shark tagging study. Our program will be integrated with programs in other parts of the world through information exchange with A.I.B.S. on shark tagging.

The prospect of recruiting additional taggers is excellent. Evidence of interest in sharks and shark fishing is reflected in the increasing numbers of sportsmen who wish to cooperate in our program and by the fact that a list of the 25 top publications sold by the Government Printing Office this year includes "Sportsman's Guide to Sharks of Northeastern U.S."

We plan to continue the shark tagging program during 1965 and if possible to expand the tagging area to cover the entire east coast.

Survey of New York-New Jersey waters

Casey's team tagged or examined over 500 sharks representing 11 species between May 19 and September 28, 1964. They landed 273 specimens on 41 longline sets. These included 142 sandbar, 38 blue, 23 sand, 13 white, 7 dusky, 5 bull, 43 dogfish and 2 mako sharks. The average catch of 2.1 sharks per 100 hooks within the study area was higher than in 1963 (.49) but lower in 1962 (7.29) or in 1961 (8.93).

On October 2nd during a tagging cruise aboard a cooperative sportsman's boat at Montauk, Long Island, Casey hooked and landed a 13' 4" female white shark. The calculated weight of the immature specimen was over 1,500 pounds, the largest fish landed on rod and reel in the Atlantic.

A report summarizing our shark studies during 1961-1964 now in preparation is scheduled for completion this winter.

SENSORY BEHAVIOR

Sound perception of bluefish

Mr. Olla developed a technique for recording electrocardiograms of bluefish while conditioning the test animal to sound stimulus. This technique provides an accurate means of determining whether a fish perceives the stimulus under study of measuring threshold values.

Although this work is still in its infancy it has established that (1) bluefish do hear, and (2) physiological recording coupled with training is a valuable tool in testing sensory system potentials in a pelagic teleost.

Feeding patterns - bluefish

Mr. Olla made systematic observations of various patterns of behavior in young bluefish (5-7" long) including general feeding behavior (using motion pictures for analysis) and effects of various light intensities on feeding and schooling responses. Also he began preliminary laboratory studies of activity cycles as related to day and night lengths approximating light cycles at the latitude of New Jersey.

In analyzing method of capturing prey and events constituting a feeding response he found that as the degree of starvation and the number of days of captivity increased, fish successively took fish which were more and more unlike the live, whole, uninjured prey they had initially preferred. They eventually ate small pieces of fish flesh and responded to solutions of macerated fish flesh in sea water.

When bright light (over 100 ft candles) was abruptly changed to dim red light (1-3 ft candles) vision was impaired and the fish no longer responded to introduced prey. During this period, before the fish's retina had become dark adapted (five to eight minutes) they no longer schooled as they had before the light change and after dark adaptation, but randomly

dispersed about the tank. Intense feeding responses were elicited within 20 seconds after introduction of macerated fish flesh solution, but the animals could not locate the prey and swam into the side of the tank. It thus appears that schooling in the bluefish, as in many other species, seems to be visually dependent and that feeding behavior patterns can be elicited by olfactory clues alone.

On the subject of light dark cycles, preliminary work indicates that there may be some cycles of activity related to time of day or night. This work will be continued in the large new behavioral tank using adult bluefish.

Vision in bluefish and mullet

Olla began studies of color vision in bluefish and mullet. All of the experimental fish conditioned readily to a visual stimulus by red or green tabs and experiments are continuing to measure degree of color discrimination.

DISTRIBUTION OF YOUNG SUMMER FLOUNDER

The first task of the DOLPHIN was to engage in the large scale cooperative study of larval summer flounder distribution, coordinated through the Atlantic States Marine Fisheries Commission. Participating in this program besides the Sandy Hook Marine Laboratory were the fishery conservation agencies of New York, New Jersey, Maryland, Virginia and North Carolina; and the Woods Hole laboratory of the Bureau of Commercial Fisheries.

The DOLPHIN first made a 7-day survey cruise from New Jersey to North Carolina with Paul Hamer of N. J. as chief scientist. At 24 collecting stations, made at 10-mile intervals along 4 transects, 126 samples of plankton were taken. Preliminary examination indicates fish eggs and larvae at most stations.

A follow-up cruise in December to study the drift of larvae to the south, was frustrated by a severe storm. Flounder larvae were taken at one out of three stations that were occupied off Atlantic City. This cruise will be completed

in January when larvae up to 8 mm long should be concentrated off Virginia and North Carolina.

To improve our ability to identify summer flounder eggs and larvae in the field, the Sandy Hook Marine Laboratory with assistance from the New Jersey Marine Fisheries Laboratory collected eggs and sperm from spawning fish caught by draggers and hatched fertilized eggs in the laboratory. Larvae lived for a maximum of 5 days past hatching.

We observed unusual behavior pattern of flounder eggs. Between the fourth and fifth days, about 12 hours before hatching, the floating eggs became sticky, adhered to each other in small clumps and sank to the bottom where hatching took place. This suggests that in nature flounder eggs change from a pelagic to demersal situation just before hatching. The hatching mortality was massive. The larvae that survived remained at the bottom of the beakers, showing little activity.

NATURAL HISTORY OF RED SNAPPER

Frank Mosely, working at the University of Texas, has been studying collections of the red snapper Lutjanus aya from the Louisiana and Texas coasts. Although his primary objective is to determine food habits, he is also including in his study growth rate and other aspects of their biology. Studies of material from the Louisiana coast are complete. Studies of material from the Texas coast will continue through March 1965. Stomach analyses of adult snapper were collected from two snapper reefs off the Louisiana coast. They reveal considerable difference in food habits between the two areas. On reef 33 located at North latitude $28^{\circ} 38'$, West longitude $90^{\circ} 34'$, 50 percent of the diet were invertebrates, of which 39.2 percent consisted of the pelagic Tunicate, Salpa confederata, 7.5 percent mollusks and 2 percent crustaceans. Fish constituted 37.3 percent of the diet and the remaining 14 percent was unidentifiable detritus. On reef 97 located at North latitude $28^{\circ} 15'$, West longitude $91^{\circ} 00'$, fish constituted 82 percent of the diet, while the remaining 18 percent was split between shrimp and crab remains.

Juvenile snapper were collected in waters from 7 to 30 fathoms. These fish ranged in size from 86 to 175 mm standard length. Stomach analysis showed that the Chaetognath, Sagitta sp., was the most common, families Penaeidae, Cragonidae, and Palaemonidae made up 26 percent of the diet, while larval crustaceans constituted 7 percent. While amphipod, Hyperia sp., which is parasitic on jellyfish was found commonly, no jellyfish remains could be identified. Fish remains made up 17 percent of the total diet, and unidentifiable detritus 14 percent.

Stomach analyses from the Texas coast are presently incomplete. Data indicate that a colonial Tunicate (the species yet unidentified) is the commonest food, while fish of the families Sciaenidae and Carangidae are also important food items. Shrimp and other invertebrates are of minor importance.

Scale samples from both juvenile and adult snappers have been collected for age-growth studies. Preliminary studies indicate that growth rings form on the scales, but seasonality of the formation of these rings, and applicability for age-growth determination need further investigation. In addition, a length-frequency graph of all snapper collected is being kept up to date for use in age-class determination.

Present data indicate that red snapper spawn in July and August in waters 30 fathoms or deeper, and that they reach sexual maturity and spawn during their first year. Although no snapper larvae have been collected, there seems to be an inshore transport of larvae, for juvenile snappers from 25 mm up were collected in early September in 9 fathoms. These juveniles remained in shallow water until late October and early November, when they began to move offshore.

EXPERIMENTS IN AQUARIUM MANAGEMENT

Experiments to measure and compare influence of artificial and natural sea water on longevity, coloration, behavior and growth of tropical marine fishes will be conducted at

The Sandy Hook Marine Laboratory in cooperation with the National Fisheries Center and Aquarium.

During the study, test media will be analysed for the accumulation of nitrates, nitrites, and ammonia under different rates of medium replacement (0-45 percent of tank volume monthly) and recirculation (0.1 to 12 times the tank volume daily). Preliminary experiments with sergeant-major (a Pomacentrid), blue-striped grunt and porkfish (Pomadysys), and cubbya (a Sciaenid) indicate that these would make desirable test species.

Progress to date includes installation of electricity, air control, temperature control, water storage, and aquarium facilities.

GENERAL

Narragansett marine game fish research laboratory

The plans for the new Narragansett Marine Game Fish Research Laboratory are finally completed after more than a year of planning, designing and revising by Regional Office engineers, architects, and staff members of the Sandy Hook Marine Laboratory. If all goes as planned, a construction contract should be awarded by early spring and construction begun by late spring or early summer with a projected completion date of one year after construction begins.

New facility for study of fish behavior

The new sea water tank for fish behavioral studies is nearing completion and should be ready for use by the end of January. The elliptical concrete tank is 35 feet long, 16 feet wide, 10 feet deep, has six large viewing windows, and will hold approximately 30,000 gallons of water. The room in which the tank is housed is temperature controlled (60 to 80 degrees F.), light proof, and sound retardant. Sea water is supplied by a new well point with an underground pipe line coming into the building adjacent to the tank area.

At the outset the tank will be used to study feeding and schooling patterns and the effects of various photoperiods on the behavior of adult bluefish.

Research vessel DOLPHIN

Congress provided \$80,000 special funds in fiscal year 1964 for the Bureau to acquire (from Government excess) and convert a ship for the laboratory. The small amount of funds that were allowed for conversion and activation work restricted our acquisition to a very modest ship. Also she would have to be in A-1 condition and completely equipped.

With such an optimistic goal we were fortunate to find an ideal ship -- an excess 107-foot Army craft, LT 1959, which had been built in 1952 and never used. She was obtained by the Bureau in December 1963 through a renewable loan agreement which included all necessary operating equipment and spare parts. She was taken out of mothballs and activated at Detyens Shipyard, Wando, S. C. during April and May. The conversion work (hull and internal changes) was separately contracted to Wilmington Shipyard, Wilmington, N. C. This work began in June and ended in September.

The DOLPHIN displaces 390 tons loaded and is powered by a single screw through a 1200 hp Fairbanks Morse diesel engine. She cruises at 12 knots and is able to range 5 to 10 days from port at a time. She carries a crew of 8 to 10 plus a scientific party of 4 or 5. At present the DOLPHIN consists of a good hull and engine but with a minimum of accessory gear. A final phase of development is necessary to prepare her for her scientific research mission.

Other floating equipment

The CHALLENGER, our 65-foot vessel, has been improved this year with the addition of an electronic fish finder and a new radar set. The deckhouse was rebuilt to provide a large working deck aft. The hull and superstructure appear strong and her machinery is in good order thus ensuring many years of good service.

A new 26-foot motor launch purchased and put into service in our Florida operation has proved to be an excellent all around boat.

Library

Edith Manning with Bori Olla as staff liaison, has reorganized parts of the library. She has instituted a new shelf system for periodicals, incorporated all unsorted periodicals into the new system. She also organized and filed the collection of maps, accessioned and listed books, decorated and made usable the reading area, bound 218 volumes of periodicals and set up display shelves for current periodicals.

As of December 31, 1964 this library owns 531 books, subscribes to 81 periodicals, receives 26 government serial publications and 11 magazines of conservation organizations. Dr. Walford conveyed as a gift to the laboratory his personal library of reprints and serials, comprising over 16,000 items.

Sea temperature workshop

Clark and Stone planned, guided and reported upon a "Workshop on Techniques for Infrared Survey of Sea Temperature" under the sponsorship of the Office of Naval Research in cooperation with the Committee for Scientific Exploration of the Atlantic Shelf. The Workshop drew 40 specialists from various parts of the country and provided the first extensive review of the modern infrared technique for large scale survey of sea surface temperatures. The report of this Workshop is now in press as a Bureau circular and will contain the complete proceedings of the Workshop as well as 12 special reports by contributors to the session.

STAFF

Dr. L. A. Walford, Fishery Biologist
John R. Clark, Fishery Biologist
Dr. Jan C. Prager, Fishery Biologist
John G. Casey, Fishery Biologist
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Daryl L. Mayberry, Administrative Assistant

TIBURON MARINE LABORATORY
Tiburon, California
Gerald B. Talbot, Director

HIGHLIGHTS

The Cooperative Tagging Program for marlin and sailfish has resulted in several returns from striped marlin tagged off southern California and Baja California.

Monthly temperature surveys of three areas of the Pacific coast in cooperation with the U.S. Coast Guard were continued, and sea surface temperature charts produced from these surveys are currently of interest to over 100 oceanographic and meteorological scientists.

Marine aquarium facilities were completed on the wharf, and 3,000 chinook salmon have been acclimated to sea water and are being reared to maturity for disease studies in cooperation with the Western Fish Disease Laboratory.

A project was initiated in June to study the important sport fishes of Yaquina Bay, Oregon.

FEEDING AND SCHOOLING BEHAVIOR OF GAME FISH

Edmund S. Hobson, who is working on this project, participated in the International Galapagos Expedition from January 6 to March 8 where he extended his studies to include species closely related to those he is studying in Baja California.

Continued study in Baja California on the grouper, Mycteroperca rosacea, has disclosed a difference in feeding behavior and in stomach contents between large (over 250 mm) and small fish. This difference is related to the difference in inshore-offshore movements of the two sizes of grouper. It was also observed that during the day the larger (over 50 mm) and smaller herring (Harengula thrissina) occupy different niches which are most favorable for their survival.

Interesting observations were made on coronetfish (Fistularia sp.) which used large groupers as shields to stalk their prey, and at "cleaning stations", where small fish removed parasites from larger fish.

ENVIRONMENTAL STUDIES

Sea surface temperatures

A total of 36 synoptic survey flights using the airborne infrared sensing unit was made in cooperation with the U.S. Coast Guard. Data from monthly flights in each of the three coastal areas were issued in the form of isotherm charts, one chart for each survey flight, and sent to approximately 100 fishery, oceanographic and meteorological laboratories and scientists who have requested this information.

Technical improvements have been made in calibration of the airborne instrument. During flight, the instrument is checked against a water bath of known temperature. A comparative IRT reading with a known sea-surface reading is made on every flight. The latter are provided by the U.S. Naval Electronics Laboratory Oceanographic tower and the U.S. Coast Guard lightvessels, and furnishes for comparison, simultaneous observations in time and space.

Water temperatures off the central California and northern Oregon and Washington coasts were cooler in the latter half of 1964 compared to 1963. Data on coastal water temperatures in comparative locations indicate average decreases of 1.4°F. for the northern area, 2.2°F. for the central area, 5°F. for the offshore area near Tanner Bank (100 miles offshore), and 2.2°F. for the Gulf of Santa Catalina.

Sea surface current studies

Drift cards sealed in plastic bags were dropped from U.S. Coast Guard aircraft once each quarter during the infrared temperature flights to obtain information on the current

ights to obtain information on the current patterns on the inshore continental shelf. About 10 percent have been returned, but returns by areas were disproportionate - 10 percent from the central area, 15 percent from the northern area, and 20 percent from the southern area.

In general, the drops in March disclosed southern drift in all three areas. In June the drift was northerly in all three areas, except the coast of Mexico where it was to the south. In September the drift was northward in the northern area, while in the southern area (drops made in October) the drift was to the south. Returns from the central area from cards dropped in September were few in number, but recoveries north of Pt. Reyes showed a southern drift, while cards dropped south of Pt. Reyes disclosed a northern drift.

Catch-temperature relationship

Sport fishing party boat operators interested in learning more about the relationship between depth, water temperature, and catch have cooperated with the laboratory by logging bathythermograph records while on fishing trips, and furnishing a log of the catches. To date, 213 such records have been obtained. These data will be analysed in the near future to determine if a relationship exists between temperature, depth, and species caught.

Bottom currents

A project to determine the feasibility of using plastic sea-bed drifters in the Monterey area and to determine cumulative non-tidal drift was completed. A report on the project is in final draft.

Interest in bottom drifters is due to their usefulness in mapping non-tidal bottom currents and drift in the spawning areas of marine fishes and determination of the relationship of drift to the migration of larval and juvenile fishes from spawning areas to the nursery areas.

Two airborne drops of 125 plastic sea-bed drifters each were made in May 1962 and May 1963. From the first drop 8 drifters

were recovered, two on the shore in north Monterey Bay 287 days after drop over the Monterey submarine canyon (depth, 500 fathoms). From the second drop pattern 18 drifters were recovered.

POPULATION STUDIES

Observations by aerial fish spotters on the abundance and distribution of pelagic schooling fishes off the California coast from Monterey to the Mexican border were continued under contract. A total of 375 separate fish spotting flights was made in 1964, and information on species, location, and abundance was recorded. Records during the first part of 1964 were made on portable tape recorders. The transcription of numerical and narrative material from tape to a coded record was found to be excessively time-consuming. As an alternative, an 8 x 11 chart of the area survey was adopted on which the pilot marks his flight track and notes any fish observations. Observations are now coded directly from the original pilot charts using the established California Department of Fish and Game block area grid designations. A code system for the data was developed and progress is being made on coding and analysing these data.

COOPERATIVE TAGGING PROGRAM

Several recoveries of tagged striped marlin (*Makaira audax*) were reported in 1964 by Japanese tuna longline vessels operating off the coast of Mexico. A total of 5 tags was recovered in 1964, indicating a northward migration along the coast of Mexico for one marlin tagged off Acapulco, and a southward migration for those tagged off the tip of Baja California and off southern California.

Late in 1964 a marlin tagged off southern California was recovered near the tip of Baja California. This is the first recovery from our program of a fish tagged in southern California waters. Continued tagging and recoveries will lead to a better understanding of the life histories of these big game fishes, including migrations, rate of growth and fishing mortality. Increased commercial fishing by Japanese longliners off Mexico assists in this project since this is the main source of tag recovery.

To accelerate the cooperative marine game fish tagging program, tagging equipment and tag return posters were distributed to many individuals, big game fishing resorts in Baja California and the Mexican mainland, and to a southern California fishing club formed for the purpose of tagging marlin and sailfish. Tag return posters in Japanese were distributed to fishing companies and laboratories in Japan to facilitate the return of marlin and sailfish tags.

Late in 1964 the Mexican Department of Fisheries (Dirección General de Pesca e Industrias Conexas de México) became an official cooperator in the cooperative tagging program and has offered assistance of its personnel and laboratories in furthering the objectives of the program.

LIFE HISTORY OF FOUR IMPORTANT FISHES OF YAQUINA BAY, OREGON

This project is being carried out on a part-time basis by graduate student Alan Beardsley at Oregon State University. It involves four of the most important species in Yaquina Bay - striped seaperch (*Embiotoca lateralis*), white seaperch (*Hanerodon furcatus*), pile perch (*Rhacochilus vacca*), and the starry flounder (*Platichthys stellatus*).

To study migration, 135 flounders and 215 perch have been tagged to date. The majority of the perch caught for tagging were females, indicating that females are either more abundant in the bay or have a different distribution than the males. Recoveries to date of perch indicated little movement from the tagging site.

GENERAL

A small sea-water aquarium has been constructed on the south wharf. It was barely completed when we were requested to attempt to rear to adults 3,000 chinook salmon fingerlings at the laboratory. These fish have experienced an episode of Sacramento River Chinook Disease, a virus, at the Coleman National Fish Hatchery. The Bureau's Western Fish Disease Laboratory desired to follow the disease during salt water adaptation and residence, and to determine the immune response.

Through experimentation it was determined that these fish required a gradual change over a period of 5 to 6 days to become acclimated to salt water. They have grown rapidly, doubling their size in 3 months. Our facilities became overtaxed as a result, and half these fish were transferred to the California Academy of Sciences' Steinhart Aquarium. Two groups of fish are now available for study.

An all steel, 65-foot "T" boat has been acquired as surplus from the U.S. Army. It will be converted to a research vessel as soon as funds are available for this purpose.

STAFF

Gerald B. Talbot, Director
James L. Squire, Jr., Fishery Biologist
Sven I. Johnson, Fishery Biologist
Edmund S. Hobson, Fishery Biologist
Charles E. Gnose, Fishery Biologist
(terminated June 30, 1964)
Alan J. Beardsley, Fishery Biologist
Walburga M. Reynolds, Clerk (Typing)
Arthur C. Madsen, Maintenceman

GENETICS

FISH GENETICS LABORATORY
Beulah, Wyoming
Walter R. Bridges, Acting Director



HIGHLIGHTS

The highlight of the year was the dedication of the planned laboratory on Tuesday, October 6. Senator Gale William McGee gave the dedicatory address; the acceptance was made by Commissioner Pautzke. The day was warm and beautiful and a surprisingly large number of people assembled at the comparatively isolated site to enjoy a barbecue luncheon, the pleasant ceremonies, and the opportunity of visiting "Branch A" and learning about the proposals for conversion to a research station.

Preliminary plans for development of the extensive facilities needed for planned studies of genetics and the selective breeding of improved trout were completed by contract with a private architectural and engineering firm. Detailed construction plans have been drawn for the extension of four existing log cabins to provide permanent quarters for resident staff.

Exteriors of existing log buildings were finished. Quarters above the garage were rehabilitated. Pumps and piping for the domestic water system were replaced and a new fire protection system was installed. The small hatchery building and a series of earthen ponds were improved and they now harbor New Zealand strain rainbow trout reared from eggs taken from three-year-old broodstock at nearby McNenny NFH.

These fish have been used in trials of new or modified fish-cultural equipment--incubation and rearing jars, a vertical-flow rearing box, and small circular tanks. Air spawning equipment and controlled temperature baths for static bioassays have been constructed and used in preliminary tests.

Two marginal parcels of land were declared surplus, one of which may be added to the holdings of the neighboring Black Hills National Forest.

Protocol has been outlined for research work units now being initiated, that completely exploit available facilities. Most effort will be expended on studies undertaken in cooperation with the Fish-Pesticide Research Laboratory, but a start will be made in establishing gene pools, inbred lines, and stocks for selection of a DDT resistant strain. Hereafter, progress of the Fish Genetics Laboratory will be reported quarterly.

STAFF

Walter R. Bridges, Fishery Biologist
Robert G. Piper, Fishery Biologist
Peter F. Smith, Fish Hatchery Manager

TECHNICAL COMMUNICATION

PUBLICATIONS, MANUSCRIPTS IN PRESS, SPECIAL REPORTS AND MAJOR ADDRESSES

- Allison, Don, Burton J. Kallman, Oliver B. Cope, and Charles C. Van Valin.
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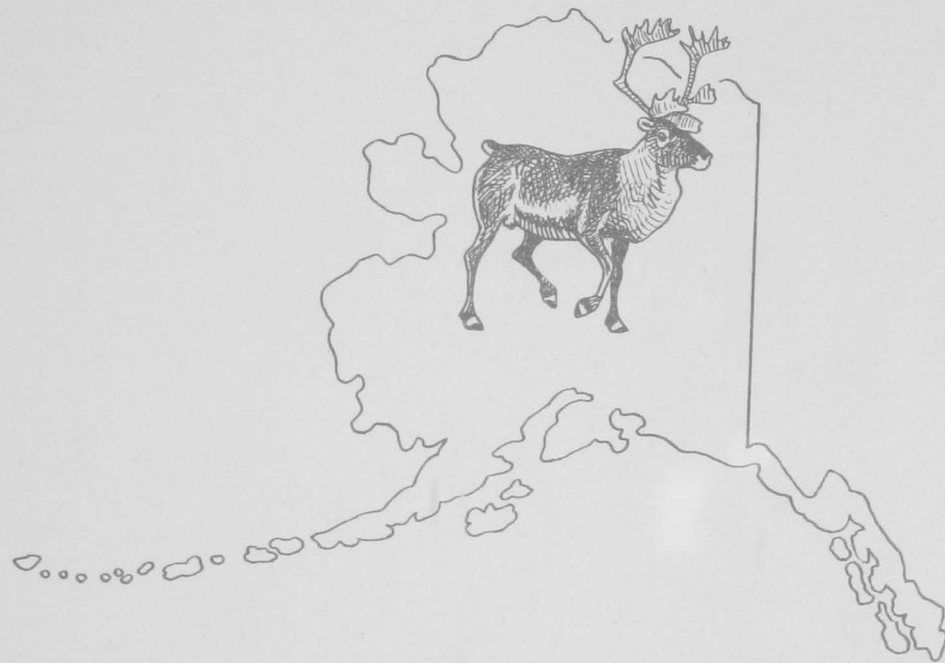
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214
211
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Distribution of Alaskan Mammals



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Library

Edith Manning with Bori Olla as staff liaison, has reorganized parts of the library. She has instituted a new shelf system for periodicals, incorporated all unsorted periodicals into the new system. She also organized and filed the collection of maps, accessioned and listed books, decorated and made usable the reading area, bound 218 volumes of periodicals and set up display shelves for current periodicals.

As of December 31, 1964 this library owns 531 books, subscribes to 81 periodicals, receives 26 government serial publications and 11 magazines of conservation organizations. Dr. Walford conveyed as a gift to the laboratory his personal library of reprints and serials, comprising over 16,000 items.

Sea temperature workshop

Clark and Stone planned, guided and reported upon a "Workshop on Techniques for Infrared Survey of Sea Temperature" under the sponsorship of the Office of Naval Research in cooperation with the Committee for Scientific Exploration of the Atlantic Shelf. The Workshop drew 40 specialists from various parts of the country and provided the first extensive review of the modern infrared technique for large scale survey of sea surface temperatures. The report of this Workshop is now in press as a Bureau circular and will contain the complete proceedings of the Workshop as well as 12 special reports by contributors to the session.

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HIGHLIGHTS

The Cooperative Tagging Program for marlin and sailfish has resulted in several returns from striped marlin tagged off southern California and Baja California.

Monthly temperature surveys of three areas of the Pacific coast in cooperation with the U.S. Coast Guard were continued, and sea surface temperature charts produced from these surveys are currently of interest to over 100 oceanographic and meteorological scientists.

Marine aquarium facilities were completed on the wharf, and 3,000 chinook salmon have been acclimated to sea water and are being reared to maturity for disease studies in cooperation with the Western Fish Disease Laboratory.

A project was initiated in June to study the important sport fishes of Yaquina Bay, Oregon.

FEEDING AND SCHOOLING BEHAVIOR OF GAME FISH

Edmund S. Hobson, who is working on this project, participated in the International Galapagos Expedition from January 6 to March 8 where he extended his studies to include species closely related to those he is studying in Baja California.

Continued study in Baja California on the grouper, Mycteroperca rosacea, has disclosed a difference in feeding behavior and in stomach contents between large (over 250 mm) and small fish. This difference is related to the difference in inshore-offshore movements of the two sizes of grouper. It was also observed that during the day the larger (over 50 mm) and smaller herring (Harengula thrissina) occupy different niches which are most favorable for their survival.

Interesting observations were made on coronetfish (Fistularia sp.) which used large groupers as shields to stalk their prey, and at "cleaning stations", where small fish removed parasites from larger fish.

ENVIRONMENTAL STUDIES

Sea surface temperatures

A total of 36 synoptic survey flights using the airborne infrared sensing unit was made in cooperation with the U.S. Coast Guard. Data from monthly flights in each of the three coastal areas were issued in the form of isotherm charts, one chart for each survey flight, and sent to approximately 100 fishery, oceanographic and meteorological laboratories and scientists who have requested this information.

Technical improvements have been made in calibration of the airborne instrument. During flight, the instrument is checked against a water bath of known temperature. A comparative IRT reading with a known sea-surface reading is made on every flight. The latter are provided by the U.S. Naval Electronics Laboratory Oceanographic tower and the U.S. Coast Guard lightvessels, and furnishes for comparison, simultaneous observations in time and space.

Water temperatures off the central California and northern Oregon and Washington coasts were cooler in the latter half of 1964 compared to 1963. Data on coastal water temperatures in comparative locations indicate average decreases of 1.4°F. for the northern area, 2.2°F. for the central area, 5°F. for the offshore area near Tanner Bank (100 miles offshore), and 2.2°F. for the Gulf of Santa Catalina.

Sea surface current studies

Drift cards sealed in plastic bags were dropped from U.S. Coast Guard aircraft once each quarter during the infrared temperature flights to obtain information on the current

ights to obtain information on the current patterns on the inshore continental shelf. About 10 percent have been returned, but returns by areas were disproportionate - 10 percent from the central area, 15 percent from the northern area, and 20 percent from the southern area.

In general, the drops in March disclosed southern drift in all three areas. In June the drift was northerly in all three areas, except the coast of Mexico where it was to the south. In September the drift was northward in the northern area, while in the southern area (drops made in October) the drift was to the south. Returns from the central area from cards dropped in September were few in number. Recoveries north of Pt. Reyes showed a southern drift, while cards dropped south of Pt. Reyes disclosed a northern drift.

Catch-temperature relationship

Sport fishing party boat operators interested in learning more about the relationship between depth, water temperature, and catch have cooperated with the laboratory by logging bathythermograph records while on fishing trips, and furnishing a log of the catches. To date, 213 such records have been obtained. These data will be analysed in the near future to determine if a relationship exists between temperature, depth, and species caught.

Bottom currents

A project to determine the feasibility of using plastic sea-bed drifters in the Monterey area and to determine cumulative non-drift was completed. A report on the results is in final draft.

Interest in bottom drifters is due to their usefulness in mapping non-tidal bottom current drift in the spawning areas of marine fishes and determination of the relationship of drift to the migration of larval and juvenile fishes from spawning areas to the nursery areas.

Two airborne drops of 125 plastic sea-bed drifters each were made in May 1962 and January 1963. From the first drop 8 drifters

were recovered, two on the shore in north Monterey Bay 287 days after drop over the Monterey submarine canyon (depth, 500 fathoms). From the second drop pattern 18 drifters were recovered.

POPULATION STUDIES

Observations by aerial fish spotters on the abundance and distribution of pelagic schooling fishes off the California coast from Monterey to the Mexican border were continued under contract. A total of 375 separate fish spotting flights was made in 1964, and information on species, location, and abundance was recorded. Records during the first part of 1964 were made on portable tape recorders. The transcription of numerical and narrative material from tape to a coded record was found to be excessively time-consuming. As an alternative, an 8 x 11 chart of the area survey was adopted on which the pilot marks his flight track and notes any fish observations. Observations are now coded directly from the original pilot charts using the established California Department of Fish and Game block area grid designations. A code system for the data was developed and progress is being made on coding and analysing these data.

COOPERATIVE TAGGING PROGRAM

Several recoveries of tagged striped marlin (*Makaira audax*) were reported in 1964 by Japanese tuna longline vessels operating off the coast of Mexico. A total of 5 tags was recovered in 1964, indicating a northward migration along the coast of Mexico for one marlin tagged off Acapulco, and a southward migration for those tagged off the tip of Baja California and off southern California.

Late in 1964 a marlin tagged off southern California was recovered near the tip of Baja California. This is the first recovery from our program of a fish tagged in southern California waters. Continued tagging and recoveries will lead to a better understanding of the life histories of these big game fishes, including migrations, rate of growth and fishing mortality. Increased commercial fishing by Japanese longliners off Mexico assists in this project since this is the main source of tag recovery.