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\* Resigned November 29, 1963.

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SPORT FISHERY RESEARCH LABORATORIES AND STATIONS  
Proposed laboratories - - - - - ○

# PROGRESS IN SPORT FISHERY RESEARCH, 1963

## INTRODUCTION

Calendar year 1963 research progress is documented in this report with highlight statements for each major area of study, more detailed back-up material, and a record of technical communications, expanded this year to include most of the important oral presentations of research findings to scientists and other conservationists. Space limitations preclude the inclusion of a great many special presentations to such groups as civic and service clubs, school science classes and clubs, and radio and television audiences.

Research administration made progress this year, too. We became full participants in the Science Information Exchange, filing currently statements of research in progress as one means of interdisciplinary communication. In the process of enrolling in the SIE, we have developed a research project and work unit system which documents the current program for administrative, scheduling and budget-planning purposes.

The annual fishery research conference was held in October at Denver. The conference included discussions of: the Cooperative Fishery Unit Program, possible effects of proposed animal care legislation on fishery research, participation in the Science Information Exchange, problems and possible solutions regarding acquisition of experimental fish, proposed plans for career development and rotation of personnel, problems encountered in starting a new research program, need for development of uniform quality in experimental fish, recent improvements in instrumentation and methodology, standardization of techniques and terminology, multiple use of talent and equipment, and needed fishery research including expansion of current program and initiation of new research. The conference was concluded with the consensus of the group, rather than formalized resolutions, concerning matters of interest to the branch.

Mid-year appropriations, for fiscal year 1964, provided moderately increased support of established research and for initiation of new research. The research program increase of \$180,000 (FY 1963, \$1,877,000; FY 1964, \$2,057,000) includes: \$55,000 for budgeted expansion of reservoir studies; \$21,000 for fish control studies; \$52,000 for fish-pesticides research; \$40,000 to employ a nucleus professional staff and maintain the property acquired for the Fish Genetics Laboratory in Wyoming, and \$9,000 to meet Pay Act costs. Included in the \$544,000 appropriated for construction are: \$100,000 for further development of the fish farming experimental station at Kelso, Arkansas; \$50,000 for engineering and architectural planning for a fish-pesticide research laboratory at Columbia, Missouri; \$75,000 for similar planning for a marine game fish research laboratory on Narragansett Bay, Rhode Island; \$132,000 for rehabilitation of a surplus vessel and certain research facilities at Sandy Hook Marine Laboratory; \$115,000 for engineering and architectural plans for the Fish Genetics Laboratory and for rehabilitation of the existing buildings at the laboratory site; and \$72,000 for construction of ponds at the Fish Control Laboratory at Warm Springs, Georgia.

One of the highlights of progress during 1963 was the acquisition on May 28 of some 885 acres along Sand Creek in northeastern Wyoming for the development of a Fish Genetics Laboratory. The area provides an excellent water supply and numerous facilities which can be economically modified and expanded to provide for genetics research. In December a contract was negotiated with a Wyoming architectural-engineering corporation for development of plans for the laboratory. Dr. James E. Wright, Jr., Professor of Genetics at Pennsylvania State College, was employed to prepare the prospectus for the fish genetics research program.

Planning for a marine game fish research laboratory at Narragansett Bay was begun in 1963.

1963 Conference Photograph  
10/7 - 10/11 Denver



1A

Front: Bruno von Limbach  
Al Swartz  
Gerry Talbot  
Paul Thompson  
Ray Johnson  
Bert Walford  
Ronald Eisler  
Fred Meyer  
Bob Jenkins  
Jim Mullan  
Tom Duncan  
Bob Rucker  
Ken Wolf  
Donald Allison  
John Buckley

Rear: Ray Hess  
Kermit Sneed  
Ralph Burress  
Chuck Walker  
Bob Lennon  
Art Phillips  
James Squire  
Neal Woodall  
John Halver  
Reed (Pete) Nielson  
Norm Reimers  
Jim Stevenson

Chuck Walburg  
Norm Benson  
Roger Burrows  
Bob Combs  
John Ross  
Pete Bullock  
Bob Piper  
Bob Bridges  
Jack O'Donnell  
Ollie Cope  
Charles Van Valin

## PATHOLOGY

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



The Eastern Fish Disease Laboratory is located on the second floor of this building. The fish hatchery and the very limited holding facilities for experimental fish are on the first floor.

### HIGHLIGHTS

A gram-negative, nonmotile bacterium, possibly a Pasteurella species was isolated from a massive kill of white perch (Roccus americanus) in the Chesapeake Bay and surrounding tidewater area.

An encapsulated, gram-negative, nonmotile rod, tentatively identified as a nonmotile variety of Pseudomonas fluorescens, was isolated from an epizootic of goldfish (Carassius auratus).

The actinomycete isolated 2 years in succession at Bowden Springs NFH was identified as Nocardia asteroides.

A symposium, "Fish Microbiology" was presented during the A.I.B.S. meeting at Amherst, Massachusetts.

By means of tests for virus, healthy spawners were selected from a lot which contained carriers of infectious pancreatic necrosis. Healthy fry have been obtained from such selected parents, and another means of control of this disease has been achieved.

A comparison of European EGTVED virus and of IPN virus has shown the two to be distinctly separate agents.

Specific immune serum of high titer was developed against IPN virus by rabbit immunization.

Studies on the development of Myxosoma sp. in the head cartilage of bluegills have been completed.

One of the black-spot trematodes, Uvulifer ambloplitis, was reared in the laboratory.

A preliminary study on Ichthyophthirius immunity has been completed.

A histological study of the developing lymphocystis cell, with special emphasis on origin of inclusion bodies, was completed.

#### BACTERIAL DISEASES

##### Aeromonas and Vibrio

Initial differentiation of these two groups of bacteria was started using the vibriostat 0/129 and Difco 0-F medium containing 1 per cent glucose. Ten cultures fermented glucose with no gas production, but only one culture was sensitive to the vibriostat 0/129. These methods proved useful in an attempt to identify cultures, pathogenic to shellfish, which were sent to this laboratory by Mr. Haskell Tubiash, Bureau of Commercial Fisheries Laboratory, Milford, Connecticut. We now have obtained additional cultures from various sources and will try additional procedures in an attempt to better separate these groups.

##### Chemoprophylaxis of bacterial fish diseases

In central European pondfish culture antibiotics are being used to protect fishes from bacterial infections. The antibiotics are injected intraperitoneally at the time of the year when fish have low disease resistance, and fish cultural practices require handling. Attempts made in this country to apply this method to bass culture were disappointing since fish injected with antibiotics apparently succumbed to the toxic effect of the drugs. To determine safe dosages and safer formulations, William Walsdorf, 1962/63 trainee, made tests to determine safe levels of all available forms of chloramphenicol injected intraperitoneally and intramuscularly into several species of fish. Preliminary tests were also made with chlor-tetracycline. He found that different formulations of chloramphenicol vary in their toxicity to fish depending on the route of introduction. He also found that several forms of the drug

were safe to the tested fish in quantities considerably greater than required for effective chemoprophylaxis. A report is being edited.

##### Nocardia asteroides in rainbow trout

The organism isolated from fingerling rainbow trout at the Bowden Spring NFH, two years in succession was identified by Dr. Ruth Gordon as Nocardia asteroides. It is stated in the 7th edition of Bergey's Manual of Determinative Bacteriology that this organism was isolated from brain and pulmonary lesions in man. This is interesting since the rainbow trout at the Bowden hatchery whirled in a tail-chasing motion. The organism was found in the brain capsule and in the abdomen which was distended and filled with a compact mass of this organism (figs. 1 and 2).

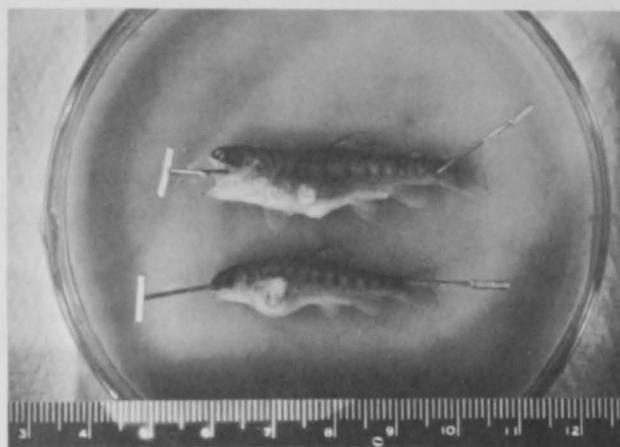


Figure 1:--Rainbow trout fingerlings from the National Fish Hatchery at Bowden, W. Va. Lesions caused by infection with Nocardia asteroides.

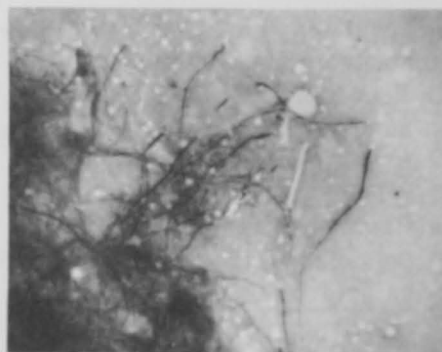


Figure 2:--Nocardia asteroides from a lesion in rainbow trout from the N.F.H. at Bowden, W. Va. 1500x.



## Corynebacterial kidney disease

James Warren, 1961/62 trainee, demonstrated a highly significant correlation of water hardness and incidence of corynebacterial kidney disease, and an attempt was made to test the relation between severity of this disease and water chemistry. Using hard spring water (2,500 ohms resistivity) and the same water diluted with deionized water to soft quality (25,000 ohms resistivity) Einar Wold, 1962/63 trainee, fed carcasses of fish which had died of kidney disease to both brook and rainbow trout fingerlings.

Contrary to expectations, rainbow trout and brook trout both became infected and died in the hard water, but only rainbows died in the soft water. It was tentatively concluded that water itself is not the principal factor causing high incidences of kidney disease under hatchery conditions, but that other factors are probably involved. A severe outbreak of kidney disease in yearling brook trout at Leetown has been in progress since December, 1963. Since Leetown is a hard water station, it is evident that hardness of water alone is not the limiting factor in the incidence of this disease.

An effective artificial medium was needed in this work and would be generally useful for research on kidney disease. Commercial Mueller Hinton medium from one source met such needs when supplemented with 0.1 percent l-cysteine HCl, but recently tested batches proved less satisfactory. Einar Wold prepared Mueller Hinton medium according to the original description. Several preparations of amino acids and of agar were tried, but the specified ingredients proved most satisfactory. When supplemented with cysteine, the medium permitted isolation of the bacterium.

## Fish kill in the Chesapeake Bay

During the summer of 1963 a very large fish kill occurred in the Chesapeake Bay and the tributaries. White perch (*Roccus americanus*) was the main victim but a considerable number of striped bass (*R. saxatilis*) also died. Due to the gradual spread of mortalities, specificity of the fish affected, and isolation of the same

species of bacterium from most of the fish which were examined, it is concluded that the bacterium isolated was not a casual contaminant but contributed to the epizootic.

The bacterium from the massive kill of white perch appears to be a member of the genus Pasteurella. This assumption is based on morphological and physiological characteristics, especially the bipolar staining nature of the organism (figs. 3 and 4). We hope to submit the bacterium for serological identification in the near future.

Although bacteria probably of the genera Vibrio and Aeromonas were isolated from species other than white perch involved in the kill, the Pasteurella-like bacterium was isolated only from white perch and striped bass. This and other points were brought out during the meeting of the Committee to Coordinate Studies of Fish Mortality in the Potomac River and Other Maryland Tidal Areas, held at Annapolis, Maryland, October 12, 1963. Dr. Eugene

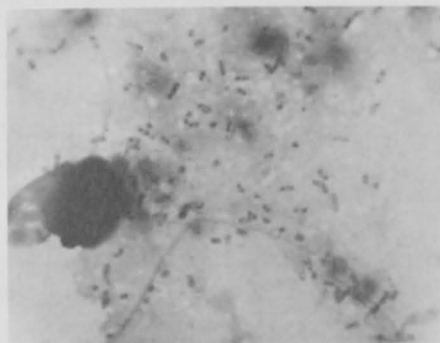


Figure 3:--Bacterium isolated from white perch. Tissue smear 1500x.

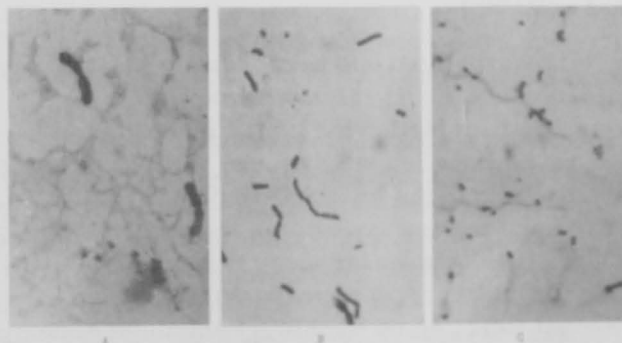


Figure 4:--Bacterium isolated from white perch grown in medium containing A) 0.5 percent NaCl; B) 1.0 percent NaCl; C) 2.0 percent NaCl.

Cronin, Chairman of the Committee, invited Dr. Snieszko and G. L. Bullock to attend the meeting in order to present the findings of the bacteriological examination of the fishes and discuss the possible role of Pasteurella in the kill.

As far as is known the kill has now completely stopped. The Committee has issued a statement of policy and recommendations concerning future fish kills and the need for study of ecological conditions in the bay area.

#### Encapsulated bacterium from goldfish

An encapsulated bacterium, isolated from an epizootic of goldfish (*C. auratus*) from a private hatchery in early 1963 has been identified as a nonmotile variety of Pseudomonas fluorescens. Although nonmotile pseudomonads are not common, the oxidative carbohydrate metabolism, fluorescent pigment production, production of cytochrome oxidase, and other characteristics place this in the Pseudomonas genus, most closely fitting the description of the nonmotile P. fluorescens.

Perhaps the most interesting characteristic of this bacterium is the capsule formation (figs. 5 and 6). Capsules can be demonstrated only in smears from infected fish. The growth on all agar media tried was slimy and viscid but in smears from these media the cells were never encapsulated. The viscid material was considered as a slime layer. Using a stain designed to demonstrate bacterial polysaccharide, both the capsular material and slime layer were found to be composed, at least in part, of polysaccharide.

The organism has been found to be pathogenic to goldfish and rainbow trout (Salmo gairdneri), by injection but not by feeding. Based on the injection studies, the bacterium was more pathogenic for goldfish than trout. External and internal symptoms of the infected fish were the same as for many bacteremias namely; petechiae, occasional accumulation of peritoneal fluid, and a flaccid inflamed intestine at times filled with a thick yellow fluid. One interesting feature noticed with the infected goldfish was the viscid material in the peritoneum

which was probably the bacterial polysaccharide. A manuscript describing the characteristics of the bacterium and its pathogenicity for goldfish and trout is in preparation.

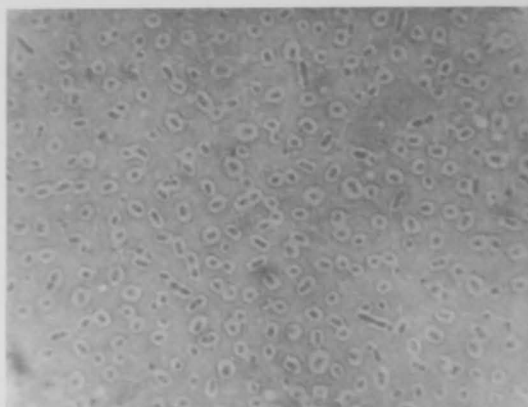


Figure 5:--Capsulated bacterium, most likely a nonmotile variant of Pseudomonas fluorescens, isolated from an epizootic in goldfish, 1500x.

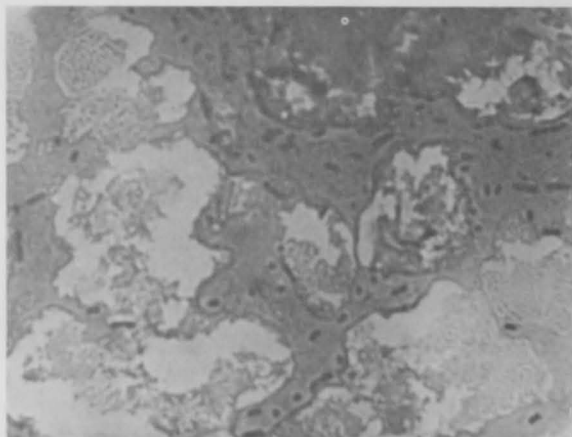


Figure 6:--Capsulated bacterium, most likely a nonmotile variant of Pseudomonas fluorescens, isolated from an epizootic in goldfish, 1500x. Capsular material is confluent producing amorphous slimy material with bacteria embedded in it, 1500x.

#### Replacement for Leetown Medium #8

Dextrose Proteose agar #3 (DPA agar) which is commercially available from Difco Laboratories, Detroit, Michigan, with addition of 10-20 mcg of filter sterilized cocarboxylase (also commercially available from many scientific supply houses) has been found to support the growth of Hemophilus piscium satisfactorily.

The growth of this bacterium is not as abundant on the DPA agar with added cocarboxylase as on medium #8 with added fish peptone. However, the medium has proved a satisfactory replacement for routine culturing and also in slab form for a storage medium. Cultures stored for nearly 2 months in this medium in the refrigerator with 2-3 week transfers have remained viable. Many strains of H. piscium initially grew on the DPA agar with no added cocarboxylase, but died after 2-3 weeks storage at refrigerator temperatures. Since components are commercially available, the medium should have wider distribution. A note for publication is planned.

#### Crystal formation in furunculosis agar

In a joint effort with A. J. Ross of the Western Fish Disease Laboratory an attempt was made to find the cause of clumps of crystals resembling bacterial colonies which appeared upon incubation in Difco Furunculosis Agar. The cause was found to be a precipitation of tyrosine which was added to the medium to enhance pigment formation.

A joint note has been submitted to The Progressive Fish-Culturist.

#### Lyophilization

Stock cultures were lyophilized this year, and through most of the lyophilization a double vial system was used. A small vial was used for the culture lyophilization and this vial was in turn sealed under vacuum in a larger vial. A single vial, constricted, and designed for lyophilization, is now used. These vials are made by the Virtis Company (Catalog No. 10-196; 1 ml. capacity) and will be supplied by Scientific Products. The vials are expensive (\$33/gross) but the time saved from the 2 vial system justify their use, at least in this laboratory.

### VIRAL DISEASES

#### Characteristics and identification of infectious pancreatic necrosis (IPN)

The consistent frank pathology which is found only in the pancreatic tissue of fishes with

IPN is a most interesting feature of this disease. Parisot and Yasutake found subtle pathology in the kidneys of some western specimens, but we have not found this in the East. We also believe that hyaline degeneration of skeletal muscle is not related to the infection, and this is at variance with the original description of the histopathology. Our belief is based on the observations that hyaline degeneration is not a consistent symptom, and that similar if not identical degeneration occurs in the absence of IPN.

These considerations prompted a search for IPN virus among other tissues. Accordingly, a series of yearling brook trout (Salvelinus fontinalis) survivors of an epizootic, was examined first by testing fecal samples and secondly by testing peritoneal washings. Feces showed 5/9 to carry virus, while 7/9 of the peritoneal washings were positive. More important was the fact that neither material revealed all the carriers. Selected tissues from the first fish to be tested were carefully removed, homogenized, and titrated in RTG-2 cell cultures. Rather surprisingly, significant amounts of virus were found in several tissues for which there was no known histopathology.

Quantities of IPN virus found in various tissues of a yearling brook trout identified as a carrier of infectious pancreatic necrosis:

Foregut	70,430 ID <sub>50</sub> /ml.*	Liver	794 ID <sub>50</sub> /ml.
Kidneys	12,590 ID <sub>50</sub> /ml.	Heart	794 ID <sub>50</sub> /ml.
Spleen	5,012 ID <sub>50</sub> /ml.	Hindgut	794 ID <sub>50</sub> ml.
Stomach	1,995 ID <sub>50</sub> ml.	Muscle	No evidence of virus
Hindgut	794 ID <sub>50</sub> ml.	Testes	No evidence of virus

\* Infectious doses for 50 percent of the units inoculated - calculated by Kärber's method.

While there is a distinct possibility that the above results may not represent the general situation, which could best be determined with a larger sample, the data support Parisot and Yasutake's findings, and our interpretation of of hyaline degeneration of muscle. The results also suggest that careful search may reveal histopathology in other tissues.



Viral hemorrhagic septicemia (INuL or EGTVED disease) is a serious problem in European trout culture. Several forms of the disease are recognized, and the symptoms are many and varied. Virus has been isolated from diseased Danish fish, but it is not yet known whether this agent is responsible for all epizootics. The whirling symptoms described for viral hemorrhagic septicemia are essentially the same as those seen in fish with IPN. We considered it essential to know whether EGTVED virus and IPN virus are one and the same agent.

Dr. Mogens Jensen, who first isolated the EGTVED agent, furnished material for comparison with IPN virus. The European virus proved larger than IPN virus, rapidly lost infectivity in glycerol, was ether-sensitive, readily inactivated by heat, attained a comparatively low titer in RTG-2 cells, and was affected by slight changes in pH; these attributes contrast with those of IPN. In addition, rabbit anti-IPN serum did not neutralize EGTVED virus.

#### Serological diagnosis of IPN

Identification of viral agents is most generally accomplished with serological methods which require use of specific immune sera. In collaboration with Microbiological Associates Inc., IPN virus was used as antigen for the production of rabbit anti-IPN sera. As measured against several hundred infectious doses of virus the various sera reached titers of from 1:1,024 to 1:8,192, and optimal titer development time was determined. Subsequently, strain LWVRT 60-1 virus was grown in serum-free cell cultures and a derived clone (ATCC #VR 299) was grown in serum-fed cells. Both antigens were supplied to the collaborator for follow-up studies.

#### Role of the male fish in transmission of IPN

We have published detailed findings on the carrier state of IPN among adult female brook trout. A subsequent test of a larger sample of the same hatchery stocks revealed the presence of virus in 3 of 15 females and in 2 of 14 males. The isolated agents were passaged until final dilution represented 10<sup>-50</sup>, or less, of the original material, and then they were tested for identity. By means of cell culture neutral-

ization tests using rabbit anti-IPN serum, all agents were identified as IPN virus. Adequate material is on hand for titrations of virus and of sera from the individual fish.

#### Control of infectious pancreatic necrosis

Therapy of true viral diseases is possible for few infections and then only under special circumstances. At present, practical control of IPN is probably limited to 1) PREVENTION-- one can avoid using eggs from sources known to have a history of the disease, or eliminate the source of the virus, and 2) IMMUNOLOGIC PROCEDURES-- vaccination may be used to avoid or to reduce mortality from the infection.

Our first work with brood stock indicated that by careful testing it was possible to identify carrier fish. We consider it likely that there will be circumstances under which the time, effort, and expense of such screening will be justified.

Immunologic procedures are effectively used in medical and veterinary virology. With particular reference to infectious pancreatic necrosis, such procedures have certain limitations. For instance it does not seem feasible to administer virus by an unnatural route of infection and thereby induce active immunity in trout fry. Nor does it seem likely that there would be time for protective antibody formation following oral administration of virus; epizootics are evident in the offspring of carrier parents soon after hatching and may in fact exact their toll throughout incubation. Although active immunity is not discounted it is considered possible that an attenuated live virus could better protect against virulent virus by the mechanism of interference.

Preventive and immunologic procedures are both being actively pursued at this laboratory.

A field test of brook trout broodstock was started for the purpose of identifying and culling carriers of IPN virus. Furunculosis and trauma from handling and branding caused severe mortality and forced early termination of the work. However brief, the attempt showed that technical problems and a high risk of accidental infection could present formidable obstacles to successful

completion of similar programs under normal hatchery conditions. Consequently, the plan was altered and the work resumed under laboratory conditions.

Brook trout spawners were tagged by our collaborator, Arthur D. Bradford of the Pennsylvania Fish Commission, and moved to this laboratory. Fecal specimens and peritoneal washings were taken and tested for virus. The early tests correctly identified 9 of the 10 fish actually used for spawning. Spermatic and ovarian fluids were tested at the time of spawning, and one supposedly clean fish was found to harbor IPN virus. Matings were made between individuals which were free of IPN virus and between those in which one or both parents were identified as carriers. All work was done carefully and every effort made to prevent cross-contamination. Eggs from each pairing were incubated in separate units of jar-type incubators. Eggs from supposedly IPN-free parents were kept in one battery, and eggs from suspect or frankly infected parents were kept in another battery.

Charles Carlson, 1963/64 trainee, assisted with all phases of the work and has had responsibility for eggs and fry during incubation and rearing.

Fry were obtained from most matings and though initial egg numbers were not determined, the results showed graduation--the greatest number of fry resulted from IPN-free parents and the least fry from a mating where both parents were infected. Where one parent was infected the hatch was intermediate. Subsequent testing of fry showed that IPN occurred only where one or both parents were identified as carriers and that IPN-free parents gave fry which remained free of infection.

Thirty-one fish were tested, but many were lost from furunculosis, and others did not ripen at a time when the eggs or sperm could be used.

The above facts showed that single testing of either fecal material or of peritoneal washings was not adequate to reveal all carriers. If the eggs from the supposedly clean but actually infected fish had been pooled with eggs from clean

fish, the usual epizootic probably would have resulted. The use of jar-type incubation successfully confined the infection to one jar, and other jars in the battery remained free of IPN.

The results also show that where necessary, brood stock can be tested and offspring can be obtained which are free of the disease. Such fish can serve as the nucleus of an IPN-free lot of brood stock and can implement one phase of control of this hatchery problem.

It is generally agreed that live virus vaccines are more effective immunizing agents than are killed virus preparations. To be effective, however, live virus vaccines must be devoid of ability to produce severe disease or death, but they must retain their specific antigenicity. Such agents are termed attenuated and are generally a result of laboratory selection for avirulence.

One strain of IPN virus has been repeatedly cloned and passed in cell cultures for the purpose of developing an avirulent form. Thus far its virulence for cell cultures has been reduced several thousand-fold, but the more important question of virulence for fish has not been tested. We intend to continue selection for attenuated virus.

#### Viral lymphocystis disease

A means of transmitting or propagating a disease is essential to its study, and the more nearly natural the method is, the more effective the research is likely to be. Lymphocystis disease has been propagated in bluegills (Lepomis macrochirus) at this laboratory for several years, but the artificial method of subcutaneous inoculation has been necessary. Several different methods were recently tried. Intraperitoneal and intracardiac injections were attempted as was the pharyngeal spray method of Dr. Weissenberg. A nine day period of feeding virus was also tried. Neither feeding nor intraperitoneal injection were effective though cutaneous lesions developed at the site of the latter. The intracardiac method was partially successful in that lymphocystis cells developed in cardiac tissue in some individuals, and these were the first internal lymphocystis cells seen by us. Weissenberg's

spray method proved highly effective, and cutaneous lesions developed over many parts of inoculated fish. The method had limited application, however, because the place of lesion appearance was not as predictable as that of subcutaneously introduced virus.

A number of workers have speculated that various parasites may serve as vectors for this infection. We investigated a monogenetic trematode but found that it did not carry infective virus from infected fish to healthy fish. Subsequently fish with lymphocystis were infected with *Ichthyophthirius* and placed with bluegills which were free of both organisms. Although the protozoan effectively infested the clean fish, it did not effectively carry the virus with it.

One factor probably involved in the high incidence of this disease among some populations is the evident predilection for certain species. Our stocks originated in a largemouth bass (*Micropterus salmoides*), and this virus has been used to infect two other centrarchids, but it would not infect a cyprinid--a species in which the disease does not occur. The pumpkinseed (*L. gibbosus*) was infected for the first time. In contrast, two lots of lyophilized virus (lesion) from the walleye (*Stizostedion vitreum*) were furnished by Dr. Walker, and used to inoculate bluegills. In no case did lymphocystis develop, and the results confirm Dr. Weissenberg's findings. We did not have susceptible walleyes to test infectivity of the material, but since we have maintained high infectivity in both desiccated and lyophilized virus from the bluegill, it was considered likely that the walleye material was infective for susceptible fishes. These results support Dr. Weissenberg's belief that the so-called lymphocystis disease in different groups of fishes represent different viruses.

Many American and recently one European researcher have asked for IPN virus for investigative purposes. Time required to fill such requests and answer the correspondence detracted from research time, therefore, it was decided to deposit IPN virus with the American Type/Culture Collection in Washington, D. C.

Most of our work has been done with an isolant taken during a 1960 epizootic at the Leetown, West Virginia NFH, among rainbow trout (*Salmo gairdneri*). This strain of IPN virus has been designated LWVRT 60-1. Subsequently the material was serially passaged 11 times in RTG-2 cells. Loss in virulence was slight, and the last passage was equivalent to a 10<sup>-60</sup> dilution of the original material. In addition clones were selected by limit dilution during the 1st, 2nd, 6th, and 7th passes. The 1st, 2nd, 6th, and 7th transfers also included filtration through Millipore HA, PH, or VC membranes. The final material was designated as strain LWVRT 60-1 Clone B2, and appropriate stocks have been deposited with the American Type/Culture Collection where it carries the designation ATCC #VR 299.

Thus far IPN virus has been isolated only from North America. The forms deposited with the virus at the American Type/Culture Collection state that distribution to foreign investigators should be accompanied by appropriate warning.

## FISH TISSUE CULTURE

### Cold blooded animal cell and tissue culture

The use of tissue culture techniques and established cell lines are convenient methods for the study of viruses, not only by fishery workers but also by other researchers. During the past year work was continued on established cell lines and attempts were made to establish new lines. RTF-1 and RTG-2 cell lines are now 4 years old, and the latter has been used in other laboratories to propagate ARBOR viruses--pathogens of man, mammals and birds. The FT cell line of frog origin is 2-1/2 years old and its description for publication is nearly complete. Some work still remains to be done on chromosome enumeration and characterization.

Franklin L. Roberts, Department of Zoology, North Carolina State College, has been a recipient of an N.S.F. research grant and was a visiting researcher at the laboratory for three months. The purpose of his visit was to learn methods of fish cell culture and to

apply them to fish cytogenetics. He used primary monolayer cultivations of cells to successfully study the chromosome complement of most of the North American centrarchids.

Cells of the amphiuma (Amphiuma means) are some of the largest known animal cells, and as such are of interest to cytologists and geneticists. Others had experienced difficulty in establishing *in vitro* cultures of such cells, therefore, we attempted to do so. Initial trials were unsuccessful but cultures were established after osmotic pressure of dispersing fluids and media was reduced from  $- .61^{\circ}\text{C}$  to  $-49^{\circ}\text{C}$ . As with our line of frog cells, a fairly high pH (7.6 - 7.8) was most favorable. Mitotic activity was maintained for weeks, but a cell line was not established.

As an economy measure, serum levels in routine cell culture media have been reduced from 10 percent to 7 percent. After a period of adaptation, cells have returned to their former activity. Because it has virtually no effect upon IPN virus, fetal bovine serum is used in most of our cell culture work. Normal calf serum has proved inhibitory, but current tests showed fractionated calf serum to be without inhibition, and the prospects are for greater economy in medium costs.

## PARASITOLOGY

### Myxosoma cerebralis

The method of infection of trout by Myxosoma cerebralis and effective treatment of infected trout are still unsolved problems. Previous attempts (1959-1961) to infect trout fry with the spores were unsuccessful. Fresh spores were placed in the stomachs of small rainbow trout fingerlings with pipettes but none of the fish became infected. An intermediate or transport invertebrate host has been suspected since 1931, therefore, spores of Myxosoma cerebralis were fed to free-living copepods and ostracods which were in turn fed to 140 rainbow trout fry. No whirling disease symptoms were noticed so the fish were autopsied and examined for spores of M. cerebralis at 8 months of age; no spores were found. We have learned by

personal communication from Russia that trout have been experimentally infected with spores that were "aged" 5 months in water prior to placing them in the stomach.

Non-infected trout fingerlings were placed in live boxes made of wire mesh (84 $\mu$  holes) and the boxes placed in the ponds at two hatcheries in Pennsylvania which had the highest incidence of whirling disease. We hoped this would exclude the assumed transport host of the disease and the fish would not become infected, indicating that a transport host is necessary. The wire mesh became so clogged that the fish suffocated. There are rumors that whirling disease has continued to spread to new locations through the transfer of infected fish.

We have collaborated with Mr. Arthur D. Bradford, Pennsylvania Fish Commission, in studies on the chemotherapy of whirling disease. Test fish were taken from a pond which had been badly infected in 1962 but unfortunately only a few diseased fish were found in the controls. The results do indicate that the test drugs tried can be used safely for fish.

### Life cycles of strigeoid trematodes

To verify the identity of the black-spot disease of fish at this hatchery, a kingfisher, the final host of the parasite, was collected, and 103 adult Uvulifer ambloplitis were collected from the intestine. The recovered trematode eggs were incubated until they hatched in 14 days. Helisoma snails were then exposed to the resultant miracidia and cercariae were recovered in 41 days. The snails produced cercariae for a period of one month and as many species of fish as were available were exposed to the cercariae. These included Lepomis macrochirus, Notropis spp., Rhinichthys spp., Semotilus spp., Catostomus spp., Salmo gairdneri, Ictalurus punctatus, Cottus sp., Etheostoma sp., Carassius auratus. In spite of the fact that black spot Neascus has been reported from most of these fish, only L. macrochirus became infected. This indicates a rather strict host specificity for the fish host. Therefore, the fish host of U. ambloplitis is the family Centrarchidae unless there are different



"strains" as we have previously demonstrated for the white grub, Posthodiplostomum minimum.

The development of U. ambloplitis in the fish was studied in bluegills at 24°C. At 3 days the metacercaria has grown very little and still looks much like the original cercarial body. At 12 days the larva has grown about 3 times as large as the cercaria and is about 300 x 140µ. The pharynx, intestinal crura, ventral sucker and holdfast organ can be seen although they are not fully developed. The two main collecting branches of the primary excretory system are prominent. At 15 days the body division into forebody and hindbody is evident. The reserve excretory channels can be seen but the spectacular calcareous corpuscles have not yet been formed. At 17 days the metacercaria is 563 x 380 µ and the suckers are well-developed. The calcareous corpuscles are present but less than half as large as a fully developed metacercariae. At 19 days the metacercaria is nearly fully developed but the tough cyst of parasite origin has not been formed. At 20 days the secretion of the cyst of parasite origin is in process; Biological studies are in process in an attempt to determine the origin of the cyst material in the metacercaria. At 22 days about half have completed the cyst of parasite origin and the metacercariae are completely developed. At 24 days 92 percent have completed cyst development.

The histopathology of U. ambloplitis in fish includes early hemorrhage and necrosis of muscle cells around the larva followed by a fibrocytic host cyst. At about 17 days black pigment cells commence to accumulate around the fibrous cyst and become more numerous during the next 10 days. Fish die when exposed to numerous cercariae.

The development was also studied at cooler temperatures. At 18-20°C the development is completed at 43 days instead of 22 days at 24°C. At 13°C the metacercaria is very little developed at 8 weeks and corresponds to the 5 day stage at 25°C. The longevity of the metacercaria is over 4 years at 12°C but probably less at warmer temperatures.

Mr. Howard Jackson, formerly manager of the NFH Senecaville, Ohio, has provided us

with some catfish fingerlings which were blinded by the eye fluke, Diplostomulum flexicaudum, or a closely related species. We hope to verify this species by rearing it to the adult stage.

A new Tetracotyle strigeoid was found in the vitreous chamber of the eye of Notropis rubellus from the Shenandoah River. The cyst of this trematode is large enough to be seen with the naked eye, and several in one eye probably cause blindness. The cysts will be force-fed to newly hatched chicks and it is expected that they will grow to adults in the small intestine.

#### Peritrichous protozoa

Dr. Jiri Lom of Czechoslovakia has compared some of our Trichodina with European species and found that (1) our two goldfish Trichodina are identical with the European forms, (2) T. fultoni from our bluegills is morphologically identical with T. domerguei f. magna Lom, 1961 of European tench and gudgeon and (3) another Trichodina of our bluegill is almost identical with a European form from Rutilus. We are hoping that further intercontinental comparisons can be made.

No further study has been made on our Scyphidia except to learn that most are Glossatella.

#### Gyrodactylus of fish

The studies on Gyrodactylus atratuli from Rhinichthys atratuli, G. margaritae from Semotilus margarita and a synopsis of Gyrodactylus of North America have been completed.

#### Myxosoma sp. in the cartilage of bluegills

The bluegills at the Leetown Hatchery are infected with a Myxosoma sp. in the cartilage which is very similar to Myxosoma cerebrealis in trout. Because of the availability of this material we have studied it extensively this year. Myxosoma-free bluegill fry were obtained from the Lamar N.F.H. to attempt an infection with the bluegill Myxosoma at Leetown. One lot was fed tissue containing spores but no fish became infected. Another lot of 60 bluegill fry was

placed in two aquaria containing a good bloom of phyto- and zoo-plankton. Many freed Myxosoma spores were then added. We had previously demonstrated that copepods and ostracods eat the spores readily. The fish were autopsied at 17-58 days but none were infected. We have nearly completed our studies on the development of the Myxosoma in the bluegill, using fry which had become infected in the ponds. The parasites were found in the cartilage only; mainly in the gill arches, other head cartilage, and occasionally fin-spine cartilage. The fry were approximately 3 weeks old when brought into the lab, and since we could not find parasites in younger fish, we presume that they become infected at about 3 weeks of age. The fish were held in parasite-free water and examined at intervals for parasite development. In 1.5 cm fish at an estimated one week post-infection, the trophozoite is 15-19 $\mu$  in diameter and contains 20 nuclei about 3 $\mu$  in diameter. At two weeks the parasite is 21 $\mu$ . At 3 weeks the parasite is 23 x 30 $\mu$  and 2 sizes of nuclei, 1.5 and 2.5 $\mu$  in diameter are present. At 5 weeks the trophozoite is 60 $\mu$  in diameter and the multicellular nature is apparent. At 7 weeks the parasite is 100-200 $\mu$  in diameter and some spores are present; the groups of nuclei, now called pansporoblasts, are apparent and 2, 3 and 4 spores can be seen in some. At 3 months the spore production appears to be complete. At 5 months the cysts are 420-790 $\mu$  in diameter, but some appear to have ruptured and the spores "leaked" out into surrounding tissue. At 7 months very few intact cysts are present, but spores can be detected in adjacent tissue, and some have apparently moved away from the cysts to points as far away as the gill lamellae. We believe many are lost to the water. The cysts become surrounded by an epitheloid granuloma which apparently does not prevent spores from "leaking" out.

The Myxosoma of the bluegill can not be the same as that of the trout. Both fish are raised at the Leetown N.F.H. and Lamar N.F.H. and trout were found infected at Lamar but not Leetown and bluegills were found infected at Leetown but not Lamar. There is also a slight difference in the size of the spores. One interesting difference in the histopathology is

the presence of eosinophilic globules in the cartilage cells adjacent to the site of infection in the Myxosoma of the bluegill.

#### Microsporidia of freshwater fish

The manuscript on Plistophora spp. of the gizzard shad and steelhead has been completed.

#### Control of Ichthyophthirius

To provide material for continued studies on Ichthyophthirius, this parasite has been successfully maintained for over one year in the laboratory. This has been accomplished by infecting previously unexposed hatchery-reared fish with harvested infective stages (tomites), obtained by placing mature Ichthyophthirius in a petri dish and leaving them until division to the tomita stage. Division time depends on temperature, with 26-27°C (79-81°F) being optimum (Bauer, O.N., 1958). Tomites are then placed in an aquarium with susceptible fish. Non-lethal to lethal initial dosages can be determined.

Experiments on controlled infection of trout with Ichthyophthirius as a method of immunization were continued with the following studies resulting. Two aquaria of a four aquarium series, were infected with approximately 500 tomites per fish, and at the first sign of visible Ichthyophthirius, one aquarium was treated with 1:6000 formalin for 1 hour every other day for 10 treatments to discourage secondary autoinfection. Another aquarium was allowed to run the course of infection without treatment, therefore, giving the parasite opportunity for secondary autoinfection. This was done to determine if the fish with a longer natural infection would develop a longer or higher degree of immunity. This did not appear to be the case as both lots of fish were refractive to the first challenge of tomites two months later. Two similar, but uninfected lots of trout served as controls; they became seriously infected upon similar challenging and many mortalities resulted. This supports Bauer's (1959) statement regarding carp immunity to Ichthyophthirius that, "the intensity of immunity results from the

strong primary attack". At the end of 7 months a second challenge of an equal number of tomites was given to the test fish and to 50 rainbow trout fingerlings serving as controls. Three fish from one aquarium and five from the other had a moderate non-lethal infection of Ichthyophthirius, while all 50 control fingerlings died within 22 days.

From these studies it appears that with controlled infection of trout with Ichthyophthirius multifiliis, a partial acquired immunity can be obtained. There is a possibility that this method of trout immunization can be utilized at hatcheries having an annual Ichthyophthirius disease problem by infecting the fish with a sub-lethal number of tomites a month or so prior to the time when the annual summer outbreak usually occurs, and after the appearance of visible Ichthyophthirius, treat the fish until all signs of Ichthyophthirius multifiliis are gone. Such fish should then be at least partially immune to the annual outbreak of Ichthyophthirius disease.

In a preliminary attempt to screen chemicals for the prolonged indefinite treatment of Ichthyophthirius disease, cysts and tomites were used as the test organism. It is hoped to find a chemical which is effective against free-living stages of Ichthyophthirius but non-toxic to fish if left in the aquarium or pond for the prolonged period of time necessary to treat the disease. Mature triphozoites were placed in small Stender dishes and the dilute chemical added. Failure of the cyst to divide or death of resultant tomites were used as the criteria of efficacy. In some instances the trophozoites in the two dishes of controls failed to develop and those experiments were discarded. The chemicals and the most dilute effective concentrations which showed promise were: ampropyroquine 0.1 ppm, possibly 0.05 ppm; quinine sulfate 2.5 ppm, possibly less; sodium chloride 0.25%, possibly less; acriflavine 3 ppm; formalin 15 ppm; Flagyl (Searle) 1.5 ppm; Roccal 0.5 ppm, possibly 0.25 ppm; Atabrine 0.25 ppm, possibly; methylene blue 2 ppm; TV-1096 (Parke, Davis) 0.1 ppm; Daraprim 0.1 ppm. Of the chemicals tested the following were not effective in the dilutions desired: Triburon (Hoffman-La Roche), Furacin, Vioform, Betadine, Lycinate, Aureomycin, Paromycin, Paa-2056 (Parke, Davis), Fumagillin and Chloroquine.

An effective systemic parasiticide would be very desirable for Ichthyophthirius because of the location of the trophozoites under the epithelium. Flagyl (Searle) has shown promise in mammalian treatment so we conducted a preliminary test on fish. Fifty 5-month-old rainbow trout were exposed to the tomites resulting from 10 mature trophozoites. The fish were then separated into 2 aquaria. Flagyl was fed in the food at a 1 percent concentration by dry weight of both to the fish in the first aquarium. The second lot served as controls and started dying of Ichthyophthirius disease in 14 days; all were dead at 23 days. Conversely, the treated fish showed less severe symptoms of "Ich" and only one died. Treatment was discontinued at 28 days during which time the fish had consumed 70 grams of food containing 1 percent Flagyl. We plan to test this further.

## HISTOLOGY

### Lymphocystis

Concurrent with the studies on lymphocystis reported in the virus section, a detailed histological study of the lymphocystis cell in sunfish has been completed. An attempt was made to identify the origin of the enormous inclusion body.

Known age specimens ranging from 24 hours to 27 days were preserved and studied in thin sections. The inclusion bodies develop about the third and fourth day and once they become visible, with the light microscope, growth is rapid. By the tenth or eleventh day a large percentage of the cytoplasmic area is taken up by these structures. In most cases the first visible granules of the inclusions are located near the nucleus. As they develop the inclusions form a ring around the nucleus, then move outward to a permanent position against the cell membrane. The inclusions are strongly positive for D.N.A. when subjected to the Feulgen reaction.

### Tissue damage by cercariae

The damage to host tissues caused by invading parasites becomes an important part of the disease process. Experimental research carried on by the staff parasitologists, is supplemented when the damage can be observed in

histological section. During the past year many specimens were sectioned and stained, both routinely and with special stains, to aid in the identification and location of parasites in the host' tissues. A manuscript describing tissue damage caused by wandering cercariae of Neogogatea kentuckiensis has been published.

### General pathology

Diagnostic histopathological examination was performed on 92 separate cases. Requests were made from several State and Federal hatcheries throughout the eastern United States. In all cases the results of the examinations have been recorded and forwarded to the respective donors. Two cases of extreme importance were the high mortality of fingerling Atlantic salmon at Craig Brook National Fish Hatchery, East Orland, Maine and the large fish kill in the Chesapeake Bay area.

### Reference slide collection

Selected stained slides from research material and specimens received for diagnostic examination, were filed in the permanent collection for future reference. These slides, especially the research material, become more important as we learn more about the pathology and histology of fishes.

## SPECIAL PROJECTS

### Electrophoresis

Dr. Joseph Hunn, recipient of a grant from the National Institutes of Health is doing research on the effects of diseases or surgical modification of internal organs on trouts. At this time studies are not sufficiently advanced for reporting of results.

### Hepatoma of rainbow trout and visceral granuloma in brook trout

Dr. Dante Scarpelli, Professor of Pathology, Ohio State University, Medical School, our collaborator, and Mr. Roger Herman, a graduate student and staff member of this laboratory, are exposing brook and rainbow trout

to different carcinogenic agents in order to study the effect of such agents on histochemistry, early histopathology and biochemistry of fish in order to develop methods for early diagnosis of these diseases. Work was started in December, 1963 and is in its preliminary stages.

### Hematological sexing of bass

Mr. Erwin Steucke, trainee in 1962/63, carried out an interesting experiment on sexing brood stock bass by hematological methods. Secondary sexual characteristics in Micropterus dolomieu and M. salmoides are small and sexing of brood fish is difficult. It is also known that female fish, as all vertebrates, are likely to contain fewer erythrocytes in blood. Examination of more than one hundred specimens by surgical and hematological methods indicated that differences in hematocrit values could be used to facilitate sexing of brood fish. A report in in preparation for publication.

### Diagnostic chart

Mr. Steucke has also prepared a wall chart for diagnosis and treatment of fish diseases. This was made at the request of Mr. Ancil Holloway, Regional Office, Minneapolis, Minnesota. It is assumed that this chart will be made generally available.

### Tissue concentrations of chemotherapeutic drugs

The U.S. Food and Drug Administration is enforcing regulations prohibiting the presence of drugs in tissues of animals to be used for human consumption. In order to determine how long different drugs are in tissues of game fishes the Branch of Fish Hatcheries is supporting work at this laboratory.

To maintain experimental fish, new tanks have been added and necessary equipment assembled. The first lots of rainbow and brook trout are being exposed to different diets fortified with sulfonamides. First results are expected early in 1964, (figs. 7, 8, and 9).





Figure 7:-- Roger Herman weighing experimental trout diet.

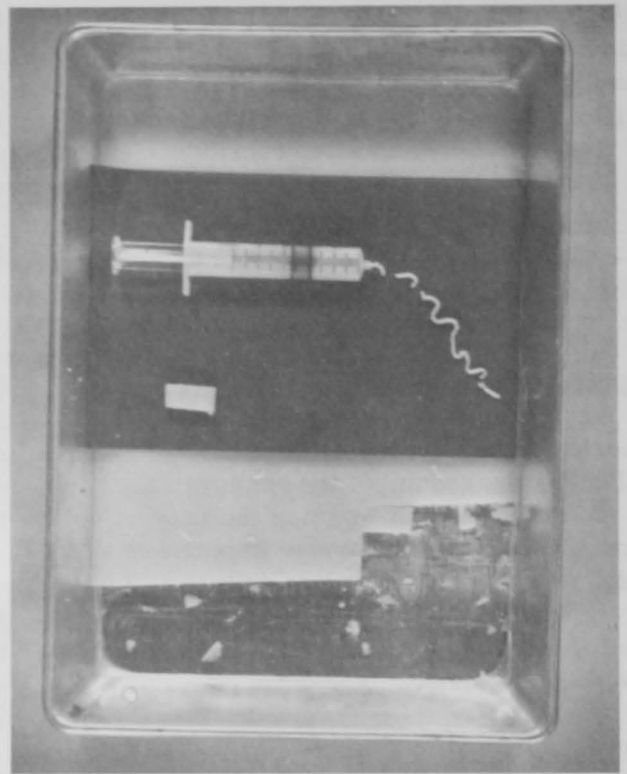


Figure 8:--Extrusion of experimental diet developed by Western Fish Nutrition Laboratory from a disposable syringe.

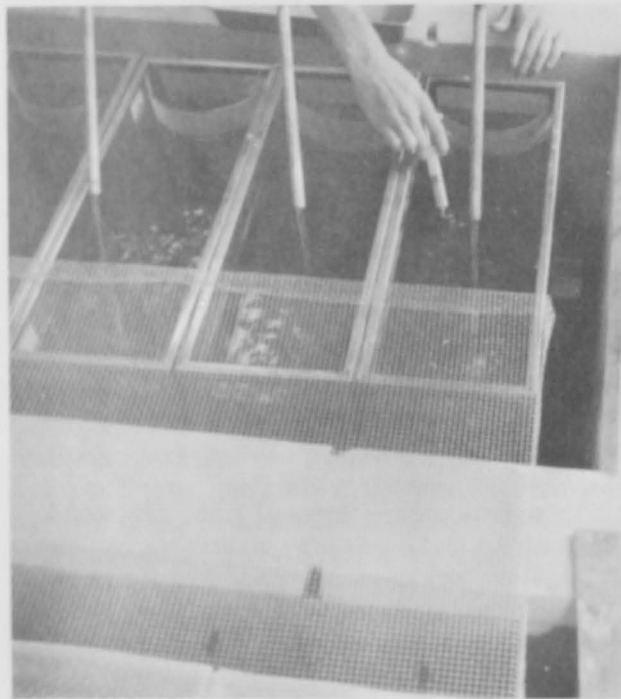
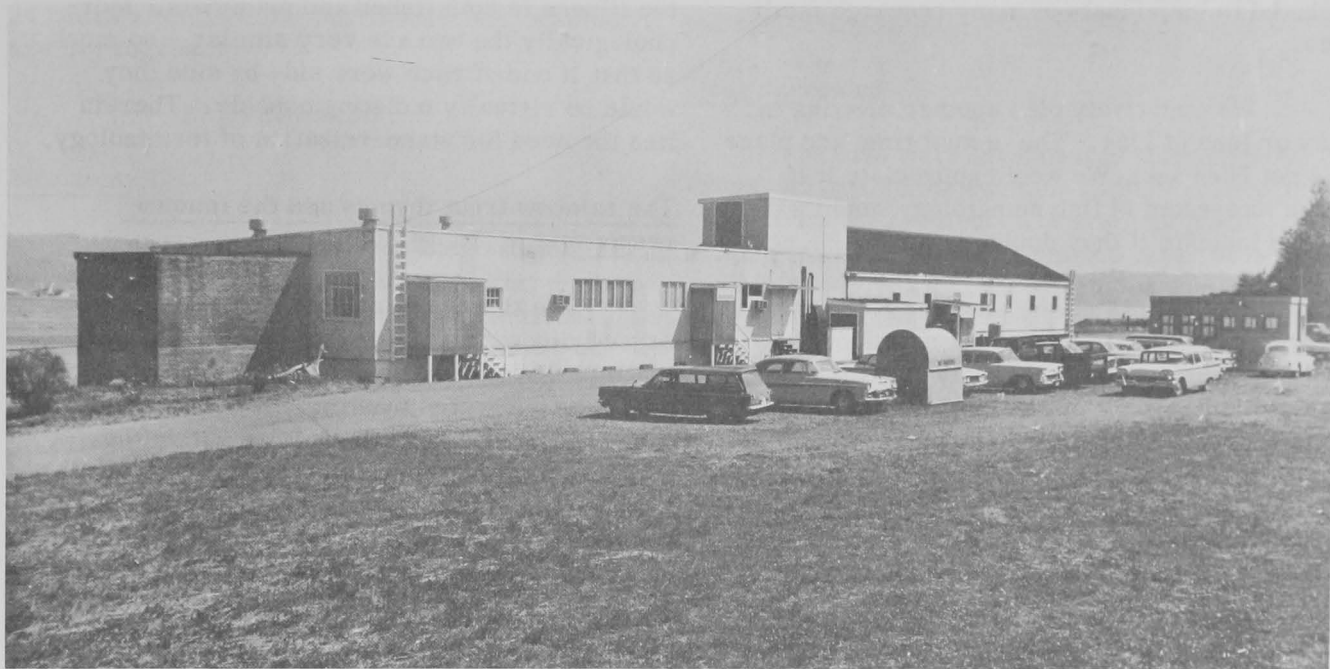


Figure 9:--Feeding trout with the synthetic diet. A syringe is used in place of a potato ricer.

#### STAFF

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### HIGHLIGHTS

The etiologic agent of a disease of rainbow trout at the Willow Beach National Fish Hatchery in Arizona was reported as Vibrio anguillarum, a marine-type bacterium. Water is supplied to the hatchery from the Colorado River.

An epizootiological study of IPN in cutthroat trout in the West has been completed.

Mild to extensive hepatomas were observed in one fish each from four of eight groups of rainbow trout raised on different commercial dry diets.

Extensive Ichthyosporidium sp. infection in rainbow trout brain tissue noted for the first time in the United States.

Copper toxicity levels for the snails Fluminicola and Oxytrema were approximated.

No demonstrable immunity was conferred upon fish exposed to cercariae of Nanophyetus.

Oral immunization of rainbow trout against redmouth disease of bacterial origin proved successful in a laboratory experiment.

### IMMUNOPATHOLOGY

#### Hematology

The hematological changes that occur during the course of an infectious disease have been only slightly investigated in fish. This is also true for the nutritional diseases but not to the same degree.

In order to achieve some measure of standardization of terminology and methods, Joe Wales of Oregon State University, Charles Smith and Dr. L. M. Ashley of Western Fish

Nutrition Laboratory, and W. T. Yasutake and Dr. G. W. Klontz of Western Fish Disease Laboratory met in September to discuss the matter. The write-up of our findings was distributed to individuals of many research facilities.

We tentatively plan another meeting in May or June of 1964. The actual time and place has not been set. We would appreciate it if those interested in fish hematology would write us and indicate if they desire to attend.

The results of our studying fish hematology were intangible in so far as infectious diseases were concerned. Only a small portion of the hundreds of slides examined were from clinically sick fish. The majority of the slides dealt with obtaining an optimum staining technique and with describing each of the types of blood cells seen in the anterior kidney, liver, spleen, thymus and peripheral vascular system.

Of all the staining methods tried, the Leishman-Giemsa in a pH 6.4 buffer system consistently gave by far the best differential qualities to the blood cells. It apparently made no difference when the smears and imprints were stained in relation to when they were obtained if they were fixed in absolute methanol after air-drying.

From preliminary observations, hematological changes occurring in rainbow trout clinically ill with an infectious process are similar to those seen in mammals. There are, however, marked exceptions. The main difference is the role in fish of the circulating macrophage in the initial inflammatory response. These cells appear to be mobilized very rapidly from the anterior kidney and to a minor degree from the spleen. After doing their "job" they are cleared out of circulation about equally in the anterior kidney and the spleen, with the liver playing a minor part.

This macrophage has been called a monocyte by many workers. We prefer to use the term "macrophage" due to its role in the inflammatory response. The mammalian monocyte functions mainly in the subacute to chronic

inflammations and often is associated with the recuperative phase of the illness. The macrophage, on the other hand, functions, as was mentioned, in the peracute to acute phase of the illness in both fishes and mammals. Morphologically the two are very similar - so much so that if one of each were side by side they would be virtually indistinguishable. Therein lies the need for standardization of terminology.

#### The rainbow trout thymus and the immune response

The thymus in rainbow trout is a bilobed organ lying within the epithelium of the dorsal juncture of each operculum with the body. The lobes are connected by a thin strand of thymus tissue only a few cells thick lying within the epithelium of the dorsal gill cavity. The thymus is surrounded by a thin fibrous capsule and is composed of epithelioid cells and lymphoid cells (thymocytes). It is reported to be stationary and does not migrate with maturity as is characteristic of the mammalian thymus. Yet, as with the mammalian thymus, it does atrophy with age.

To test the hypothesis that since the rainbow trout thymus as a lymphoid organ participates in the immune response, two groups of 6 to 8-inch trout were given a particulate antigen (killed redmouth organisms) subcutaneously in the region of the left lobe of the thymus. One group had not been exposed to the antigen previously and the other group had been orally immunized against redmouth and furunculosis. A third group, the control, received saline in the region of the left thymus. At periods of 1, 3, 5, 24, and 48 hours after inoculation, one fish from each group was killed and duplicate imprints of the right and left lobes of the thymus, the spleen, the anterior kidney, and the liver were made. The imprints were stained with Leishman-Giemsa and with the methyl green-pyronin stains.

The results, though not conclusive, were indicative that the thymus did respond to antigenic stimulation. In the 1- and 3-hour post-inoculation samples, the left lobes of the thymus had a mitotic activity in the large lymphoblasts

and a marked increase in pyroninophilia. The control samples revealed none of these changes nor did the right lobe of the thymus in the test fish. This response was no longer manifest at 24 hours after inoculation. Subsequent studies are in progress.

## BACTERIOLOGY

### Taxonomy

Vibrio anguillarum was incriminated as the etiologic agent of a serious bacterial disease of rainbow trout at the Willow Beach National Fish Hatchery, Arizona. The organism is unusual in a freshwater hatchery as it is normally associated with diseases of fish in a marine or brackish environment. Our isolate exhibited a definite requirement for sodium chloride in culture media. The organisms failed to survive for 24 hours in tap or distilled water, but remained viable for at least 5 weeks if sodium chloride were added to the water.

A peritrichously flagellated bacterium was isolated during an epizootic of rainbow trout at Willow Beach National Fish Hatchery. This has been identified as similar to the bacterial pathogen causing redmouth disease in Idaho. This is the first time, to our knowledge, this organism has been associated with diseased fish outside of Idaho.

A culture prepared by Dr. Mark Keyes (Marine Mammal Biological Laboratory, Seattle) from a moribund California sea lion (Zalophus californianus) was submitted for study and was classified as Mycoplasma sp. This diagnosis was subsequently confirmed by the Washington State Department of Agriculture Laboratory. This is believed to be a host record for this genus of bacterium.

### Mycobacteria

An experiment concerned with the possible introduction of mycobacteria into salmon eggs at the time of fertilization was terminated at the end of four months due to gas bubble disease. Silver salmon eggs had been fertilized in the presence of a mycobacterial culture sus-

pension. Samples were examined periodically by direct smear and histopathologically for evidence of infection with acid-fast bacilli. Mycobacteria were never observed either in the egg or fry stage.

### Drug Screening

Methods were investigated whereby new drugs and antibiotics could be rapidly screened for anti-bacterial effects. A gradient plate technique was explored in which a layer of agar containing a given amount of drug or antibiotic was allowed to solidify in a square petri plate slanted just sufficiently to form an inclined plane. After placing the plate in a normal horizontal position, another layer of agar was poured containing 10 times the amount of drug or antibiotic used in the lower layer.

During subsequent incubation the diffusion of the drug theoretically results in its dilution proportional to the thickness ratio of the two agar layers, thus establishing a uniform concentration gradient. If bacterial suspensions are streaked over the agar surface parallel to the axis of the concentration gradient, the length of the growing streak should be a direct measure of the minimum inhibitory concentration. A number of cultures can then be tested on a single plate. Comparisons with serial dilution tube tests indicated a close correlation, but all drugs or antibiotics may not lend themselves to this method due to differences in diffusion coefficients.

## VIROLOGY

### Infectious pancreatic necrosis (IPN)

This virus disease accounted for much of the activity of this section during most of the year. After making the initial identification of the disease in the West late in the previous year, we conducted an intensified study to discover its source and mode of transmission. The results indicated that one or more commercial egg sources were involved in the spread of the disease in rainbow trout, and two strains of Montana cutthroat trout were the reservoir and transmitting agent for that species.

## Chinook lateral line syndrome (CHILLS)

Another disease entity of possible virus etiology was investigated during the spring and summer. An agent was isolated in chinook tissue cultures and was passed in chinook salmon up to five inches in length. Further studies of the agent will be deferred until chinook fingerlings are available for comparative studies.

## Sacramento river chinook disease (SRCD)

The virus causing SRCD in chinook salmon at Coleman NFH is being studied for its chemical composition. In addition, further management studies are being conducted to help hatchery personnel control the disease. It should be noted that the activation temperature of 56° F. reported in the recently revised Fishery Leaflet No. 562 should have read 46° F. The former temperature is near the upper limit of activity.

## Tissue culture

A rainbow trout gonad tissue culture has been established for one year and has been subcultured 18 times. It will be studied for various characteristics during the year and will be made available to interested laboratories.

## Diagnostic services

Material was processed for numerous Federal, State and private hatcheries. IPN was demonstrated in a few instances, but viruses were not demonstrable in the majority of cases.

One result of this work has been to increase our awareness of various toxic agents. It is quite possible that tissue cultures can be used to detect small amounts of insecticides or other substances suspected to be the cause of mortalities. This will be investigated during the coming year.

## PATHOLOGY

### Hepatoma

The experiment initiated in the spring

of 1962 at the Hagerman NFH to compare the effects of commercial dry diets on rainbow trout was continued. Tissues from this, the third in a series of similar experiments, were processed and examined microscopically throughout the year. Eight groups of rainbow trout were reared on eight different diets, and samples were taken when the fish were 12, 14, 16, and 18 months old. In a few cases which grossly exhibited hepatomatous nodules, all major tissues were fixed and histologically examined for possible metastasis, otherwise only the livers were examined. As in the two previous studies, all specimens were received by us without identity as to the diet. Although no basophilic nodules were observed in the 12-month tissues, one liver of this group exhibited an eosinophilic nodule. One liver from each of the 14- and 16-month-old materials also exhibited well-defined basophilic nodules. Of the 18-month-old fish, mild to extensive hepatomas were observed in one fish from 4 of the 8 diet groups. Although the other fish did not show any microscopic hepatomatous lesions, diffuse and bizarre changes as well as extensive ceroid pigment deposits were observed. The significance of these ceroid pigments in fish tissue is not clearly understood. It has been suggested, however, that they may be associated with the excessive accumulation of unsaturated fatty acids, and this may induce a lack of biologic anti-oxidants in fish under certain circumstances such as in diseased conditions or dietary deficiencies.

No metastasis was seen grossly or microscopically in any of the samples. Reports of our histopathological findings have been submitted to the Regional and Central Offices.

Liver samples from the hepatoma survey conducted by the Branch of Fish Hatcheries were received for histological examination from Ennis, Hagerman and Leavenworth NFH's. Twenty-five trout on regular production diets (dry pellet) from each of the above hatcheries were sacrificed and grossly examined by field biologists. Only those livers suspected of hepatoma were fixed in Bouin's and sent to this laboratory for confirmation. In all, 13 rainbow trout livers from Ennis, 1 rainbow trout liver from Hagerman, and 7 cutthroat



trout livers from Leavenworth were received, processed and examined. Ten, none and four respectively, were found to have hepatoma.

### Extrarenal hemopoiesis

During routine histological examination of experimental and diagnostic materials over a number of years, lymphoid areas have been observed in numerous livers of salmonids. It has been suggested that these areas are possible foci of secondary or ectopic hemopoiesis. In order to explore this possibility, an experiment was initiated this year.

Ten fish were bled one percent of the total body weight once a week. The most efficient method of obtaining blood was by direct heart puncture. Hematocrit and differential blood smears were taken at each bleeding from each of the fish. After eight weeks no appreciable changes were noted. It was surmised that the rapid recovery capability of the fish was too great to produce any notable changes with 7-day intervals between each bleeding. Subsequently, 20 fish were used for the second phase of the experiment. From these fish approximately 0.3 percent of total body weight was bled three times weekly. After the sixth bleeding the hematocrit value dropped from 46.3 to 26.9 percent. Notable changes in the differential smears began to appear in subsequent samples. Liver, spleen, heart and kidney tissues of mortalities were Bouin-fixed for histological examination. The histological picture of the mortalities indicated hyperactivity of the kidney and spleen as evidenced by the presence of numerous primitive and immature forms of both the erythrocytic and leucocytic series. Abundance of macrophages in the liver appeared to be indicative of stress factors; however, there seemed to be no appreciable increase in the lymphoid areas. The above findings seem to suggest that the liver may not necessarily serve as an organ of secondary hemopoiesis, but as a tertiary site. The spleen may be the area of secondary hemopoiesis. As soon as "normal" rainbow trout become available this coming year, the experiment will be repeated. Imprints of tissues from the mortalities will be taken in addition to the other tests mentioned above.

### Cryostat

The histochemical study of white spot disease with the use of the cryostat has been handicapped by the lack of white spot material. In addition, difficulties were encountered in preparing the yolk-sac fry for sectioning. Last month, with the cooperation of the Western Fish Nutrition Laboratory, excellent materials from their Cu-Zn toxicity studies were collected, and either quick frozen in liquid nitrogen-isopentane or fixed in Bouin's. These specimens will be used in the coming year.

The cryostat seemed to be effective as a rapid diagnostic tool. Formalin, Bouin's and liquid nitrogen-isopentane methods were also explored with some encouraging results. However, further refinement of various techniques now employed must be studied before definite conclusions can be drawn.

### Infectious pancreatic necrosis (IPN)

Histopathology was used to confirm IPN in rainbow trout from two hatcheries in Arizona and Idaho. Morphological changes in these fish appeared somewhat atypical and suggested possibly a more chronic type of infection accompanied by fibroblastic repair. In the Idaho fish extensive striated muscle hyaline degeneration was noted in all the samples. To date, very little of this type of pathology has been observed in fish from typical epizootic materials.

### Chinook lateral line syndrome(CHILLS)

In the late spring and early summer of this year, samples were received from many State and Federal hatcheries. No clinical symptoms were apparent other than the moribund state of the fish. The hemorrhagic condition was observed only after the skin was peeled back along the lateral line. (This condition was called lateral Line Hemorrhagic Syndrome. However, at the annual Fish Disease Conference held at Corvallis, Oregon, this fall, it was agreed to name it Chinook Lateral Line Syndrome (CHILLS) since this disease appears to be species-specific). A slight hemorrhage was noted at the base of the pectoral or pelvic fins in several of the fish. Histopathologically, little to extensive petechial

and hemorrhagic areas were seen in the striated muscle. In the earlier stages of the disease, little or no pathology was seen; however, in the severe cases, affected areas were congested with red blood cells undergoing pyknosis, karyorrhexis and karyolysis (Fig. 1). Adjacent to these blood-congested areas, hyaline degeneration and necrosis of the striated muscle was frequently observed (Fig. 2). No other pathological changes were noted.

Although consistent mortality was maintained in the fish injected with millipore-filtered filtrate, no significant pathology was exhibited by the moribund fish from this experimental group.

### Diagnostic services

Numerous diagnostic materials from various Federal, State and private agencies in Arizona, California, Idaho, Nevada, South Dakota, Utah, Washington and Wyoming were received, processed and examined this year. It is noteworthy to mention a group of fish which was called to our attention by the Food and Drug Administration. These were yearling rainbow trout from a private trout farm in Idaho which exhibited extensive spine curvature. Gross and histological examination revealed severe *Ichthyosporidium* sp. infection, particularly in the brain tissue (Fig. 3). Liver, kidney, heart, muscle and spleen were also involved, although the infection was not as extensive as in the brain. To our knowledge, this is the first report in the United States of brain tissue involvement with this organism resulting in scoliosis, lordosis, and kyphoscoliosis.

## PARASITOLOGY

### Studies on snail-borne fish parasites

Snails of the genera *Oxytrema* and *Fluminicola* occur abundantly in many Washington, Oregon and California streams and may often be co-resident in a given environment. Special interest attaches to these gastropods because of their role as vectors of several digenetic trematode parasites of salmonid fishes.

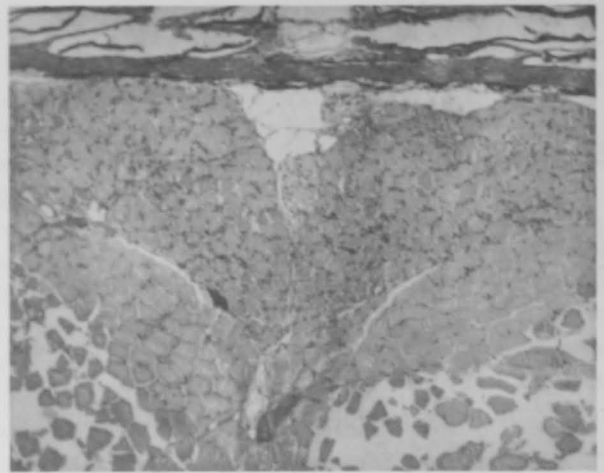


Figure 1:--Hemorrhagic areas between the muscle bundles along the lateral line from chinook fingerling with CHILLS. X-section H & E X40

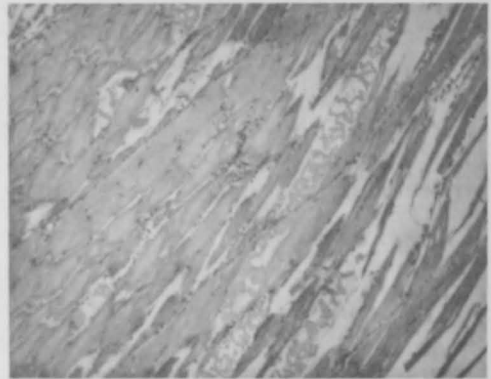


Figure 2:--Extensive muscle necrosis of the dorsal striated muscle from chinook fingerling with CHILLS. Sagittal section H & E X40

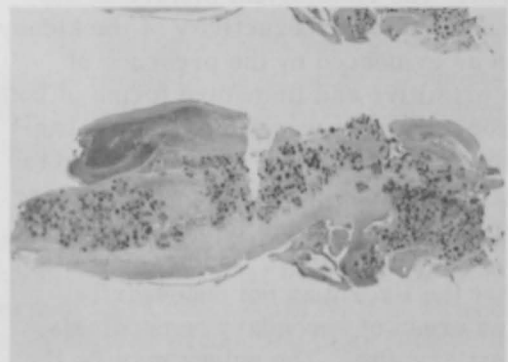


Figure 3:--Sagittal section of rainbow trout brain showing an extensive *Ichthyosporidium* sp. infection with scoliosis. Dark ovoid areas are spores of varying sizes. Periodic acid stain X16.

Ecological studies suggest that these snail populations may be particularly vulnerable to chemical control during late summer periods of low flow with attendant concentrations of snails in preferred sites. Standing-water bioassays of the effects of trace levels of zinc and copper were conducted using heavy-metal-free water and micro concentrations of the sulfates of these metals. Zinc ion concentrations of .02, .04, and .06 ppm were apparently harmless during a 14-day test period. Copper ion concentrations of .01, .02, and .03 ppm were tested for a thirty-day period; it was found that the median tolerance limit of Fluminicola was less than .01 ppm and that of Oxytrema was less than .02 ppm. These findings indicate that sustained trace levels of copper ion might be used effectively in natural environments to eliminate Oxytrema and Fluminicola with negligible effects upon resident fish and invertebrate populations. Preliminary analyses of water samples from selected stream habitats suggest that the geographical distribution of Oxytrema and Fluminicola may be limited, to a large degree, by naturally prevailing levels of one or more of the heavy metals. Difficulty of analysis of trace levels of heavy metals led to adoption of ion-exchange techniques for concentration of water samples from various major trout and salmon habitats in western Washington. Correlations are being sought between presence or absence of snails, fish species and abundance, and heavy metals content.

Previous experiments conducted at this laboratory have shown quantitatively that metacercariae of the salmon poisoning fluke, Nanophyetus salmincola, may induce significant mortality in juvenile rainbow trout at levels which are observed in naturally infected fishes. In a follow-up study, a 10-week experimental challenge of previously exposed and parasite-free fishes revealed that no protection or immunity is afforded fishes which have sustained previous infection. Accumulation of parasite burden in these two groups increased uniformly and comparably when held under identical conditions of exposure to Nanophyetus cercariae.

Additional risk to wild salmonids from snail-borne infectious agents was found in the discovery of larval Echinochasmus milvi in wild,

juvenile steelhead trout. This parasite, an echinostomid trematode, was found to induce extensive gill pathology in naturally infected fish from an environment sustaining both Oxytrema and Fluminicola populations. Our studies of these fish have shown them to be triply infected at times with Nanophyetus salmincola, Sanquinicola davisi, and the aforementioned Echinochasmus milvi. These findings, together with collective knowledge of the separate effects of these three parasites, suggest the probability of a high operating mortality rate from the combined effects of these parasites. It is likely that this condition of multiple infection occurs elsewhere in other salmonid populations. All of these parasites invade fish hosts by direct penetration of body and oral surfaces. Aside from direct effects on the host fish, the cumulative wounding action of repeated cercarial invasions provides a constant array of access sites for secondary microbial infections. The potentiation of viral, bacterial, and mycotic diseases by these parasites seems highly probable.

The studies briefly reported here indicate the salient relation of disease interaction between molluscan and game fish hosts. Feasible methods for elimination or suppression of the implicated snail vectors seem amenable to development with appropriate interest and support.

#### Studies on lowland lake trout parasites

A survey of parasites of rainbow trout from three western Washington lakes was continued through the sixth month of a proposed 12-month study. Monthly recaptures of fish planted disease-free in June exhibited the following array of parasites: protozoan gill parasite, Trichophrya sp.; larval cestodes, Diphyllobothrium sp., Ligula intestinalis, and Proteocephalus ambloplitis; and acanthocephala, Neoechinorhynchus rutili. Diphyllobothrium larval infections are associated with visceral hemorrhage, ascites, and connective tissue adhesions; In addition, a number of larvae have been found encysted within the pericardial cavity. Although co-existing bass and rainbow trout populations occur in two lakes, only one currently evidences the P. ambloplitis problem. Infections of P. ambloplitis larvae are concentrated in the liver where excessive damage is manifest. Feeding experiments have been set up to determine:



1) the vulnerability of bass to P. ambloplitis larvae for which rainbow trout have served as second intermediate host, and 2) the ability of rainbow trout to serve as definitive hosts for P. ambloplitis larvae found in bass and/or rainbow trout. Parasite availability, as determined by mean number of parasites per fish, has shown no increase since October; this observation probably reflects the autumnal decline of copepod populations which serve as first intermediate hosts for both Diphyllbothrium and P. ambloplitis.

## INTERSECTIONAL STUDIES

### Bacteriology and Immunopathology

#### Oral immunization of rainbow trout against redmouth

Rainbow trout were orally immunized against the etiologic agent of redmouth disease by feeding a pelleted diet containing killed bacterial cells. These fish, in addition to controls, were subsequently challenged intraperitoneally with virulent homotypic cultures in doses ranging from 1 LD<sub>90</sub> to 40 LD<sub>90</sub>. Only 12 percent of the immunized fish died while 81 percent of the control fish died.

In November a hatchery trial was begun at the Hagerman NFH to field test the method of orally immunizing rainbow trout against redmouth. This trial is comprised of three sections: 1) the test section which is being fed the bacterin-containing feed; 2) the treated control section that is on regular feed and will receive therapy in the event of an outbreak of redmouth; 3) the untreated control section that is on regular feed and will receive no therapy in the event of an outbreak of redmouth. In each section there are two groups. One group contains 200 pounds of fish per unit of water and the other group contains 100 pounds of fish per unit of water. The unit of water is one-half the hatchery deep tank with a flow of ca. 45 gpm at 58° F.

The test section has received the redmouth bacterin in their feed on a daily basis for two weeks, initially, and weekly thereafter. As of December 31, 1963, six weekly feedings

of the bacterin had been given. On the basis of the data obtained in the original laboratory trial, we plan to have the first challenge one week after the eighth feeding.

Coincident with the Hagerman trial is a second laboratory trial run on a much smaller scale in this laboratory. The first challenge here will be given the week preceding the Hagerman challenge. In both cases we plan to challenge intraperitoneally with 5-10 LD<sub>90</sub> of the homotypic virulent redmouth organisms.

#### Anti-redmouth and anti-furunculosis agglutinins in rainbow trout resulting from oral immunizations

In conjunction with our attempts to stimulate the production of an actively acquired immunity to redmouth and/or furunculosis in rainbow trout, ten fish from each test group were bled and the sera tested against the bacterins in question. During the course of our original trials the fish were not tested for the presence of circulating agglutinating antibodies as we felt this would introduce a stress not encountered in the routine hatchery environment.

In the case of the redmouth-immune fish the presence of anti-redmouth agglutinins was quite pronounced, with none appearing in the control fish. However, the fish fed the furunculosis bacterin and the control fish (not fed any bacterin) had demonstrable circulating anti-furunculosis agglutinins. There was, nonetheless, a quantitative difference between the immune and non-immune groups.

## VIROLOGY AND IMMUNOPATHOLOGY

#### Infectious pancreatic necrosis virus (IPN) precipitins

Four rabbits were injected intramuscularly with IPN of rainbow trout tissue culture origin plus a modified Freund's adjuvant. Several injections were given over an extended period of time. Serum samples obtained at regular intervals during the inoculation period were tested for the presence of anti-IPN precipitins using the immuno-diffusion technique.

In all but the very early samples there was a complexity of lines of precipitation due to the myriad antigens in the tissue culture medium. In order to detect the specific IPN-anti-IPN band(s), the technique of specific inhibition of precipitation as described by Bjorklund was used. In this, a particular antigen-antibody complex is inhibited or prevented from forming the visible aggregate (precipitate) by the addition in excess of the antigen in question to the diffusion medium. That is, to preclude the appearance of the specific tissue culture-anti-tissue culture precipitation bands, a calculated excess of tissue culture fluid (without IPN) was added to the diffusion medium. Thus, when the observations were made, the only lines of precipitate in the agar plate were those antigen-antibody complexes not in the tissue culture-anti-tissue culture medium, i.e., supposedly the only different antigen(s) was the IPN. There were, in fact, two faint lines in the inhibition test, but, at this point we have no conclusive evidence that they are indeed the specific IPN-anti-IPN complex. The final proof will come with subsequent testing of other sources of the virus.

#### IPN serology

A serological study of the western strain of IPN was initiated. Rabbit anti-IPN serum was prepared and neutralization tests conducted. Results indicated that neutralizing antibodies were produced against our agent and protection of tissue cultures was provided by the antiserum.

#### Cytopathology of virus diseases

Pathology studies were conducted with four viruses including IPN, SRCD, CHILLS, and OSV, a virus from sockeye salmon obtained from Oregon State. Fish and tissue cultures were infected with each of the agents and both systems were sampled at regular time intervals to determine histopathologic or cytopathologic changes. Results are currently being interpreted.

#### Miscellaneous

Preserved specimens of chinook salmon received from Australia exhibited pathology similar to that of CHILLS. This is of extreme interest as these fish were developed from Coleman NFH eggs. Fresh samples and background information have been requested so we can attempt to isolate the agent and compare the epizootic with the disease in this country.

#### GENERAL

#### Building Development

Construction on the unfinished area of our building was completed. This provides laboratory and office space for immunopathology, fish food preparation room, a conference room, additional storage space and extends wet laboratory facilities.

#### STAFF

Dr. Robert R. Rucker, Fishery Biologist  
Mr. Joseph R. Uzmans, Parasitologist  
Mr. Avron J. Ross, Bacteriologist  
Mr. Thomas J. Parisot, Bacteriologist  
Mr. William T. Yasutake, Histologist  
Dr. George W. Klontz, Serologist  
Miss Colleen A. St. Clair, Laboratory Technician  
Mr. Gary R. White, Fishery Aid

Mrs. Sarah H. Hayduk, Parasitologist  
Mrs. Gail R. Dryer, Clerk-Stenographer  
Mrs. Janice E. Martin, Physical Science Aid  
Mrs. Claudia K. Jenes, Laboratory Technician  
Mr. Garth A. Culver, Fishery Aid  
Mr. Dennis E. Crouch, Fishery Aid  
Mrs. Nellie H. Nickels, Clerk-Stenographer  
Mr. Paul D. Rogers, Fish Hatcheryman helper  
Mr. Wilmer N. Morgan, Janitor

# NUTRITION

## EASTERN FISH NUTRITION LABORATORY

Cortland, New York

Arthur M. Phillips, Jr., Chief



Eastern Fish Nutrition Laboratory, Cortland , New York

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

Brown trout, fed a Cortland pelleted food as a complete diet for a two-year period, produced eggs equal in quality to those from fish fed a meat-meal mixture.

Trout were able to utilize fat calories substituted for a portion of a standard diet but were unable to utilize the calories provided by white dextrin.

An orally administered progesterone compound reduced the growth of brook trout,

but no significant effects were found in chemical composition, blood coagulation times or gonadal development.

The blood calcium of female brown trout increased with the approach of spawning. No such changes were found in the males.

p-Amino benzoic acid exaggerated the effect of a folic acid deficiency as measured by erythrocyte count and microhematocrits.

Grading failed to increase the growth of rainbow trout. These results are similar to those reported previously for brook and brown trout.

Fish under stress were benefited by intermediate levels of dissolved sodium chloride.

Brook trout converted inorganic dietary phosphate into organic compounds (nucleic acids and phospholipids) but even four days after feeding most of the phosphorus was in an inorganic form.

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

##### Development of a complete dry food for trout

These experiments, begun in July 1961 with 3 to 4 inch brown trout, were concluded with the spawning of the fish in early November, 1963. Observations upon growth, survival, and conversion rates were concluded the first part of July at the end of two years.

During the experiment three types of pelleted food were fed to duplicate groups of brown trout as complete foods. The pellets were prepared in our laboratory-type mill. The No. 1 pellet contained 1,250 calories per pound and 43 percent protein; the No. 2, 1,105 calories per pound and 37 percent protein and the No. 3 pellet 880 calories per pound and 32 percent protein.

At the end of two years all three pelleted foods had produced excellent growth rates, good conversions, and permitted normal survival of the fish. There were no statistical differences in the performances of any of the pellets. In terms of calories and protein required to produce a pound of fish, however, the No. 3 pellet was the most efficient. It required 1,847 calories and 300 grams of protein per pound of fish produced compared to 2,099 calories and 263 grams of protein for the No. 2 pellet and 2,274 calories and 354 grams of protein for the No. 1 pellet. The low-calorie, low-protein pellet was more economical in terms of flesh produced without sacrifice of growth or efficiency of food conversion.

Periodically throughout the two-year period the trout did not appear in satisfactory condition. There were occasional lesions upon

the surface of the fish and we feared excessive mortalities would develop. Such mortalities did not occur and gradually the fish overcame their poor appearance and by the end of the experiment did not appear significantly different from the other fish held at the hatchery.

These pellets were as satisfactory as any that have been fed at this laboratory and warrant further testing at other stations.

The reproductive products were of satisfactory quality. The highest percentages to the eyed-egg stage and hatched fry were observed with eggs spawned from adults fed the No. 3 pellet (low calorie-low protein). The percentage eyed for this group was 88.0 and the percentage hatched 85.9. These results compare with 81.0 percent to the eyed stage and a hatch of 79.4 percent for eggs taken from fish fed the No. 2 pellet and 79.7 percent to the eyed stage and a hatch of 76.6 percent of eggs taken from adults fed the No. 1 pellet. The eggs from the fish fed the No. 3 pellet were larger (401 to the ounce) than those from fish fed the No. 2 or No. 1 pellet (430 and 423 per ounce, respectively). Since there were over 100,000 eggs in each group spawned from more than 60 females, these results and values are representative. The average percentage of eggs to the eyed stage taken from trout fed a meat-meal mixture during the same two-year period was 88 percent. The No. 3 pellet was as efficient as the standard hatchery diet.

The fry resulting from eggs spawned from adults fed the No. 3 pellet have been transferred to the Lamar NFH, Pennsylvania and arrangements have been made to feed the No. 3 mixture in forms suitable for smaller fish.

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

Because of observations that possible vitamin deficiencies were occurring in fish fed the previously described pelleted fish foods, three new pelleted foods were designed in which the levels of niacin, vitamin B<sub>12</sub>, and ascorbic acid were doubled in the vitamin mixture; the carrier of the mixture changed from dried skim



milk to wheat middlings; two vitamin mixtures were prepared in which one consisted of all the vitamins except choline and the second contained only choline; and an anti-oxidant (santoquinn) was added to both vitamin packages. Cottonseed meal was replaced by de-hulled soybean meal.

The No. 4 pellet is low protein (32 percent), low calorie (875 per pound); the No. 5 low protein (32 percent), high calorie (1,024 per pound), and the No. 6 is a high protein (43.6 percent) and high calorie (1,184 per pound) pellet. The calories were increased in the No. 5 pellet by the addition of a stabilized fish oil.

At the end of 6 months all pelleted foods were producing satisfactory growth and survival rates. There were no statistical differences among the experimental groups.

In terms of food conversion the No. 6 pellet was most efficient with an average value of 1.34 pounds of food per pound of fish flesh produced. This was followed by No. 5 pellet with a conversion of 1.49 and the No. 4 pellet with a conversion of 1.67.

In terms of calories, the No. 4 pelleted food was the most efficient, producing flesh at the rate of 1,461 calories per pound of gain and the No. 6 pellet the least, requiring 1,568 calories. A similar relation was observed for the production requirement for protein. The No. 4 pellet produced a pound of fish for each 245 grams of protein but the No. 6 pellet required 265 grams. The No. 5 pellet was intermediate between these values for both the calories and protein production requirement. Thus, even though the No. 6 pelleted food was the most efficient in terms of pounds of food required per pound of fish produced, it was the least efficient in terms of calories and protein, the truer measures of nutritional efficiency.

To date, none of the disorders or undesirable characteristics observed following the feeding of the previously reported pelleted foods, have appeared among the experimental fish. For the same period of time (6-months) the conversions produced by these pellets are more efficient and the growth rates greater

than those of the trout fed the previously-described pellets.

These experiments will continue until reproductive products are obtained from the adult fish.

#### Utilization of fat and carbohydrate as calorie sources in the diet of brook trout

The reduction of the calorie content of brook trout diets by the inclusion of various levels of inert cellu-flour reduced the growth rate of the fish. The replacement of the removed calories with corn oil increased the growth rate to values near those obtained with the control diet. Body analyses indicated that much of the increased growth was in the form of deposited body fat and therefore not true growth. The utilization of white dextrin to replace the calories failed to increase the growth rate of the fish and there was no indication that the dextrin was utilized by the fish.

These results are similar to those reported in last year's experiments. Changes in sources of dietary protein and/or the ratio of protein calories failed to improve the results. We believe that these results and those of previous experiments are caused by poor quality protein that cannot be used for growth purposes or the inclusion of an excess of calories over the requirement for maximum growth under our experimental conditions. Further experiments are warranted in which the amino acid ratios are altered in lieu of increasing or decreasing total protein with an inert material.

#### Effect of corn oil and a stabilized fish oil on lake trout growth rate

Lake trout were held in the cold water supply (8.3 ° C.) and fed the standard hatchery diet; the standard hatchery diet in which inert cellu-flour replaced a portion of the calories; the calorie-reduced standard diet with added corn oil; or the calorie-reduced standard diet with added stabilized fish oil. The experiments were run for 20 weeks.

The reduction of the calorie content of the diet from 724 to 464 calories per pound reduced

the growth rate of the fish by approximately one half (555 percent to 223 percent). The replacement of these calories with either corn oil or stabilized fish oil restored the growth to near that of the control (535 and 547 percent respectively). Chemical analyses of the fish's body however, showed that the increased gain resulting from the addition of either oil, was the result of doubling the fat content of the fish's body (5.2 and 5.5 percent fat in fish fed the oil fortified diets and 2.6 for those fed the control diet). The lake trout did not use the fat calories to supply energy for increased growth of the fish.

It is possible that the cold water temperature precluded the use of the added fat calories. It is probable, in view of the growth rate of the control fish, that the removal of the calories, largely in the form of protein, altered the protein quality to an extent that the fish could not utilize the available protein for growth. The oil, therefore, merely provided excess calories that were deposited in the body as a reserve energy source.

There was no difference in the utilization of the two sources of fat by the lake trout.

#### CHEMICAL COMPOSITION OF HATCHERY AND WILD TROUT

##### The effect of water temperature upon changes in chemical composition of developing brown trout eggs

Brown trout eggs were incubated at two water temperatures (8.3 and 10°C.) and samples of eggs taken after the lapse of a similar number of temperature units at the two water temperatures. The eggs were analyzed for protein, fat, ash, and water content and the results compared to determine differences in chemical changes at the two rates of development.

No differences were found in either the fat or ash content of the two lots of eggs. The water content of the eggs held at 10°C. was significantly greater than that of the eggs held at 8.3°C.. The protein content of the eggs held in the warmer water was significantly less than

that of the eggs held in the colder water. The differences in protein content are correlated with changes in the water content of the egg for on a dry basis no such protein differences were found between the two groups of eggs. Other chemical differences might be measurable if the range between the high and low water temperature could be increased. It is possible that method sensitivity was not sufficient to measure the small chemical differences that might occur at the range of temperatures used in this experiment.

##### Effect of an oral progesterone steroid compound upon growth, chemical composition, and blood properties of brook trout

Fingerling brook trout were fed a synthetic diet containing 6-methyl-17 acetoprogesterone at the daily rate of 1 milligram per pound of fish for 20 weeks. Changes in body weight, microhematocrit and blood coagulation values, gonadal development, and chemical composition of the fish were compared to their controls (no dietary progesterone compound).

Fish fed the progesterone compound had body weights and microhematocrits values lower than those of the controls. Average terminal body weights were 35.9 for those receiving the compound and 39.3 for their controls. Average microhematocrit values for the two groups were 34.3 for the compound-fed fish and 40.7 for the controls. Differences for the two experimental groups in blood coagulation times, chemical composition, or gonadal development were not significant.

#### CHEMICAL COMPOSITION OF TROUT BLOOD

##### Determination of monthly changes in the blood protein of yearling trout

The total serum protein of the blood of mature brook trout was determined at monthly intervals over an 11-month period. Serum proteins varied by season, increasing at spawning time and during the spring growth period and decreasing prior to spawning and through the winter months. These changes could be correlated with increasing or decreasing metabolic

activity of the fish. The increases and decreases occurred without respect to sex and there were no significant effects caused by diets.

#### Monthly changes in the blood calcium of spawning age brown trout

Several years ago it was reported that female codfish have a higher concentration of blood calcium at spawning time than male codfish. To determine if such changes occurred in brown trout blood, serum calcium was measured at monthly intervals over ten months.

There was a difference in the blood calcium of brown trout that was correlated with sex at spawning time. The blood calcium was higher in the females than in the males during this period but at other times of the year no sex difference in blood calcium was noted. This difference may be related to evolution as suggested by some to the earlier writers. The increase in blood calcium may also be correlated with the serum protein vitellin, which reportedly has calcium linked to its molecule and both the calcium and the protein are eventually used in developing fish eggs. The latter explanation seems reasonable because increased blood levels of unbound calcium in the ionic form might cause an osmoregulatory stress.

#### Effect of acclimation to two water temperatures on the blood properties of brown trout upon their transference to a lower water temperature

A study was conducted to continue the investigation of the effect of change in water temperature on the microhematocrit and prothrombin values in yearling brown trout.

One approach explored the possibility that the temperature at which fish are acclimated affects the response of the two properties when the fish are placed in a lower temperature water.

When two groups of fish were transferred to a water temperature of 1.5° C. for 40 hours, trout held at a constant temperature of 8.3° C. for 49 days before experimental use exhibited an average decrease of 9.5 percent in microhematocrit values whereas fish held at variable

water temperature averaging 11.° C. prior to their use showed a 30.9 percent decrease. Prior temperature conditioning did have an effect on the microhematocrit values of the fish blood.

No such difference was found in the prothrombin times of the fish held at the two water temperatures (8.3° C. and 11.1° C.) before their exposure to a water temperature of 1.5° C.. Blood plasma from the majority of fish from both groups failed to clot at this lowered water temperature unless stimulated by additional calcium ions. Clotting failure precluded the measurement of possible differences.

A second approach explored the possibility that a stress syndrome arising from handling and new environmental conditions is responsible for osmotic upsets which, in turn, result in the previously reported changes in microhematocrit and prothrombin values when trout are transferred to a lowered water temperature. This study indicated that fish that had been held at a temperature of 1.5° C. for 40 hours had microhematocrit values that were no higher than those of fish held at the same temperature for only 15 hours. Similarly, prothrombin time reactions by the fish held for 40 hours at 1.5° C. were no more favorable than those of fish held for only 15 hours in colder water. Data from this study indicate that holding fish for an additional 25 hours at the new, lower water temperature does not promote a return to normal of these blood properties.

#### Effect of dietary sulfaguanidine on blood coagulation, microhematocrit, growth, and hemopoietic tissue of immature brook trout

A 20-week study designed to investigate further the effect of dietary sulfaguanidine on blood properties of brook trout fingerlings verified the results reported for our 1962 study. The average bi-weekly microhematocrit for fish fed sulfaguanidine was 36.6 percent compared to 41.8 percent for fish fed diets lacking the chemical. Average blood coagulation times were 67.0 and 49.3 seconds for the sulfaguanidine-fed trout and the controls, respectively. These differences in microhematocrit and blood coagulation times were highly significant.

Tissue imprints made from the anterior portion of the kidney of fish from the two groups to study possible effects of sulfaquanidine on hemopoiesis did not reveal apparent differences in numbers of immature erythrocytes.

Differences in growth between the two experimental groups were not significant.

#### VITAMIN REQUIREMENTS OF TROUT

##### Effect of dietary p-amino benzoic acid and folic acid on erythropoiesis and growth of immature brook trout

At the end of a 20-week experimental period, the microhematocrit values were 40.0, 34.5, 35.0, and 31.3 percent for trout fed a complete synthetic diet, synthetic diet minus p-amino benzoic acid, synthetic diet minus folic acid, and a synthetic diet minus both p-amino benzoic acid and folic acid, respectively. Periodic erythrocyte counts verified the microhematocrit values. After the sixth week, microhematocrit values declined more consistently for trout fed the diet lacking both vitamins than did the values of the blood of trout fed the other vitamin deficient diets. A deficiency of p-amino benzoic acid exaggerated the effect of a folic acid deficiency.

No statistically significant differences could be established for growth in any of the experimental groups.

##### Effect of age, fish size, and season on the pyridoxine requirement of brook trout

The previously reported correlation of pyridoxine sensitivity of brook trout to season rather than fish size or water temperature, failed to be supported by the last series of experiments. This represents the first failure of this correlation out of a total of six tests. There is some indication that the preparation of reproductive products during the immediate pre-spawning period and the onset of spawning had an effect in these experiments.

##### Toxicity of vitamin A to trout

At the end of a 20-week experimental

period, there was no indication that 3,000, 30,000, or 300,000 units of vitamin A as a palmitate resulted in toxicity when fed to fingerling brook trout. A slight difference in total percent gain over the 20-week period was not statistically significant. Liver tissues have been sent to another laboratory for vitamin A analyses but these results have not been completed. The possibility exists that vitamin A as a palmitate is poorly utilized by brook trout; this possibility will be investigated.

#### EFFECT OF PHYSICAL FACTORS ON GROWTH OF HATCHERY TROUT

##### Effect of grading on weight gained and survival of rainbow trout

The grading of rainbow trout into two size groups the first 10 weeks of the experiment and into three size groups the last 10 weeks, failed to show increased growth rates and survival or more efficient conversions in comparison to ungraded populations. The total conversion for the graded rainbow trout was 2.57 for the 20-week period and 2.50 for the ungraded populations. These conversion factors support in part the contention that the levels of food fed were comparable for the two groups of fish. These results are similar to those previously reported for brook and brown trout.

##### Effect of water entry on the growth and efficiency of food conversion of lake trout

Because of results reported by the State laboratory at Bellefonte, Pennsylvania, experiments were conducted to compare the conventional water intake in troughs with an intake of water along the trough bottom through a perforated pipe. At the end of 20 weeks the differences found in the growth rates of these two experimental groups were not statistically significant. The average total gain was 619 percent (618 and 620 for the replicates) and 556 percent (544 and 568 for the replicates) for the perforated pipe water entry and the head entry respectively. Perhaps additional replicates, a longer test period or the running of the experiments in the warmer water supply of this laboratory would produce significant differences. There was no differences in the body chemistry, survival, or the conversion rates of the two groups of fish.



activity of the fish. The increases and decreases occurred without respect to sex and there were no significant effects caused by diets.

#### Monthly changes in the blood calcium of spawning age brown trout

Several years ago it was reported that female codfish have a higher concentration of blood calcium at spawning time than male codfish. To determine if such changes occurred in brown trout blood, serum calcium was measured at monthly intervals over ten months.

There was a difference in the blood calcium of brown trout that was correlated with sex at spawning time. The blood calcium was higher in the females than in the males during this period but at other times of the year no sex difference in blood calcium was noted. This difference may be related to evolution as suggested by some to the earlier writers. The increase in blood calcium may also be correlated with the serum protein vitellin, which reportedly has calcium linked to its molecule and both the calcium and the protein are eventually used in developing fish eggs. The latter explanation seems reasonable because increased blood levels of unbound calcium in the ionic form might cause an osmoregulatory stress.

#### Effect of acclimation to two water temperatures on the blood properties of brown trout upon their transference to a lower water temperature

A study was conducted to continue the investigation of the effect of change in water temperature on the microhematocrit and prothrombin values in yearling brown trout.

One approach explored the possibility that the temperature at which fish are acclimated affects the response of the two properties when the fish are placed in a lower temperature water.

When two groups of fish were transferred to a water temperature of 1.5° C. for 40 hours, trout held at a constant temperature of 8.3° C. for 49 days before experimental use exhibited an average decrease of 9.5 percent in microhematocrit values whereas fish held at variable

water temperature averaging 11.° C. prior to their use showed a 30.9 percent decrease. Prior temperature conditioning did have an effect on the microhematocrit values of the fish blood.

No such difference was found in the prothrombin times of the fish held at the two water temperatures (8.3° C. and 11.1° C.) before their exposure to a water temperature of 1.5° C.. Blood plasma from the majority of fish from both groups failed to clot at this lowered water temperature unless stimulated by additional calcium ions. Clotting failure precluded the measurement of possible differences.

A second approach explored the possibility that a stress syndrome arising from handling and new environmental conditions is responsible for osmotic upsets which, in turn, result in the previously reported changes in microhematocrit and prothrombin values when trout are transferred to a lowered water temperature. This study indicated that fish that had been held at a temperature of 1.5° C. for 40 hours had microhematocrit values that were no higher than those of fish held at the same temperature for only 15 hours. Similarly, prothrombin time reactions by the fish held for 40 hours at 1.5° C. were no more favorable than those of fish held for only 15 hours in colder water. Data from this study indicate that holding fish for an additional 25 hours at the new, lower water temperature does not promote a return to normal of these blood properties.

#### Effect of dietary sulfaguanidine on blood coagulation, microhematocrit, growth, and hemopoietic tissue of immature brook trout

A 20-week study designed to investigate further the effect of dietary sulfaguanidine on blood properties of brook trout fingerlings verified the results reported for our 1962 study. The average bi-weekly microhematocrit for fish fed sulfaguanidine was 36.6 percent compared to 41.8 percent for fish fed diets lacking the chemical. Average blood coagulation times were 67.0 and 49.3 seconds for the sulfaguanidine-fed trout and the controls, respectively. These differences in microhematocrit and blood coagulation times were highly significant.

Tissue imprints made from the anterior portion of the kidney of fish from the two groups to study possible effects of sulfaguanidine on hemopoiesis did not reveal apparent differences in numbers of immature erythrocytes.

Differences in growth between the two experimental groups were not significant.

#### VITAMIN REQUIREMENTS OF TROUT

##### Effect of dietary p-amino benzoic acid and folic acid on erythropoiesis and growth of immature brook trout

At the end of a 20-week experimental period, the microhematocrit values were 40.0, 34.5, 35.0, and 31.3 percent for trout fed a complete synthetic diet, synthetic diet minus p-amino benzoic acid, synthetic diet minus folic acid, and a synthetic diet minus both p-amino benzoic acid and folic acid, respectively. Periodic erythrocyte counts verified the microhematocrit values. After the sixth week, microhematocrit values declined more consistently for trout fed the diet lacking both vitamins than did the values of the blood of trout fed the other vitamin deficient diets. A deficiency of p-amino benzoic acid exaggerated the effect of a folic acid deficiency.

No statistically significant differences could be established for growth in any of the experimental groups.

##### Effect of age, fish size, and season on the pyridoxine requirement of brook trout

The previously reported correlation of pyridoxine sensitivity of brook trout to season rather than fish size or water temperature, failed to be supported by the last series of experiments. This represents the first failure of this correlation out of a total of six tests. There is some indication that the preparation of reproductive products during the immediate pre-spawning period and the onset of spawning had an effect in these experiments.

##### Toxicity of vitamin A to trout

At the end of a 20-week experimental

period, there was no indication that 3,000, 30,000, or 300,000 units of vitamin A as a palmitate resulted in toxicity when fed to fingerling brook trout. A slight difference in total percent gain over the 20-week period was not statistically significant. Liver tissues have been sent to another laboratory for vitamin A analyses but these results have not been completed. The possibility exists that vitamin A as a palmitate is poorly utilized by brook trout; this possibility will be investigated.

#### EFFECT OF PHYSICAL FACTORS ON GROWTH OF HATCHERY TROUT

##### Effect of grading on weight gained and survival of rainbow trout

The grading of rainbow trout into two size groups the first 10 weeks of the experiment and into three size groups the last 10 weeks, failed to show increased growth rates and survival or more efficient conversions in comparison to ungraded populations. The total conversion for the graded rainbow trout was 2.57 for the 20-week period and 2.50 for the ungraded populations. These conversion factors support in part the contention that the levels of food fed were comparable for the two groups of fish. These results are similar to those previously reported for brook and brown trout.

##### Effect of water entry on the growth and efficiency of food conversion of lake trout

Because of results reported by the State laboratory at Bellefonte, Pennsylvania, experiments were conducted to compare the conventional water intake in troughs with an intake of water along the trough bottom through a perforated pipe. At the end of 20 weeks the differences found in the growth rates of these two experimental groups were not statistically significant. The average total gain was 619 percent (618 and 620 for the replicates) and 556 percent (544 and 568 for the replicates) for the perforated pipe water entry and the head entry respectively. Perhaps additional replicates, a longer test period or the running of the experiments in the warmer water supply of this laboratory would produce significant differences. There was no differences in the body chemistry, survival, or the conversion rates of the two groups of fish.

Utilization of dissolved chloride by brook trout

HCl<sup>36</sup> was used to label chloride concentrations of 5, 50, 500, and 5,000 ppm in spring water. Fingerling brook trout were held through four days in these labeled waters at temperatures of 2.2, 10.5, or 18.8° C.. Absorptions of chloride were measured to determine the repression or stimulation of mineral exchanges in trout caused by relatively abrupt alterations in environmental temperature.

The fish were taken from hatchery troughs (water temperature of 8.3° C. and a chloride content of 5 ppm) and conditioned to their new environments for 24 hours before being netted into water containing the Cl<sup>36</sup> labeled sodium chloride. Previous experiments at this laboratory have shown that these environmental changes, along with netting of the fish, cause osmotic stresses. As part of the design of this experiment, an additional stress was placed on the fish by maintaining them in restricted volumes of aerated water.

Mortalities occurred at all three water temperatures when the fish were held in the two lowest chloride concentrations. Only the fish held in the 5,000 ppm chloride concentrations survived for four days in the warm (18.8° C.) water. All of the fish in both this and in the 500 ppm concentration survived for four days in the cold (2.2° C.) water, while half the fish held in the two lowest chloride concentrations died. The survivors appeared distressed. During their first two days in the 2.2° C. water, the fish in the 500 and 5,000 ppm chloride concentrations also appeared distressed, but they eventually recovered equilibrium and resumed normal opercular movements. The fish in the 5 and 50 ppm concentrations did not begin to show distress until after two days in the 2.2° C. water.

The distress and mortalities of the fish were related to their absorption and exchange of mineral ions, as measured with the labeled chloride in the water. Under stress, a more rapid exchange of the chloride of the medium with that of the body fluids was required. The

greater supply of salt in the higher experimental concentrations limited the deleterious effects of the temperature and osmotic stresses. Because of increased metabolic activity in warm water, insufficient mineral ions were available to the fish from the three lower concentrations, even though the rate of chloride uptake by the fish was markedly increased.

As observed previously at this laboratory, cold water only temporarily depressed the osmotic regulatory functions of brook trout. After this depression which lasted approximately two days in this experiment, the fish adjusted to the higher chloride concentrations (500 and 5,000 ppm), that had originally distressed them, by an increased rate of chloride absorption. Prior to this adjustment, that could be called acclimation, the chloride content of the higher concentrations osmotically distressed the fish in cold water. The resumption of a more normal osmotic regulation after two days in the cold water was also evident in the chloride exchange from the two lower chloride concentrations. After this time these fish also increased their rate of chloride exchange. There was, even in cold water, insufficient chloride in both the low concentrations to satisfy the metabolic requirements of fingerling brook trout. Delayed distress and mortalities resulted.

The combination of temperature, osmotic, and handling stresses proved fatal to these small fish. The presence of relatively low sodium chloride concentrations (only 0.5 percent chloride) permitted increased ion exchange with the environment and apparently contributed to the survival and acclimation of the trout.

Distribution of dietary phosphate to the muscles and skeleton of brook trout

To further investigate the significance of the unusually efficient and active absorption of dietary phosphorus by brook trout, p<sup>32</sup> labeled phosphate was incorporated into four synthetic feeds that were capsulated and force-fed to fingerling fish. Two levels were prepared (0.5 and 4.0 milligrams of phosphorus per capsule), either with no dietary calcium or with an amount of calcium equal to the amount of phosphorus (a 1:1 calcium-phosphorus ratio). The fish were

held, one to an aquarium, for either 1, 2, or 4 days after force feeding one capsule to each fish. Water temperature was a normal 10.5° C. and water in the aquaria was changed daily. To measure the degree of utilization of inorganic phosphorus in the structural growth of trout, skeletons of these fish were sampled for incorporation and retention of labeled phosphorus and their muscle tissues were fractionated to determine incorporation of inorganic dietary phosphorus into muscle protein, phospholipids, and nucleic acids. Additional samplings were made of labeled phosphorus contents of the skin and visceral tissues.

Generally, as previously established, the phosphorus was more rapidly and efficiently absorbed from the calcium-free diets. The recoveries of labeled phosphorus in both skeletal and muscular tissues was proportional to absorption of the phosphorus from these feeds. Incorporation of phosphorus in the bone was not reduced when calcium, present in the aquarium water, was lacking in the capsulated feed.

Recoveries of labeled phosphorus in bones and muscle tissue indicated a nearly equally efficient utilization of phosphorus from both dietary levels, with slightly more of the phosphate from the higher level being lost through more rapid excretion. With all four feeds, dietary phosphate was rapidly distributed to, and then retained in skin and visceral tissues.

Fractionation of muscle tissues showed that nearly all the labeled phosphorus was still in an inorganic form, even four days after feeding. Generally less than a tenth of the dietary phosphorus in muscles had been incorporated into organic phosphorus compounds at this time. Most of this labeled organic phosphorus was found in the nucleic acid and phospholipid fractions. The quantity of labeled phosphorus in both fractions increased from the first day after feeding. Relatively little dietary phosphate had been incorporated into muscle protein even four days after feeding.

These fish received no additional food during the four days of exposure to labeled food, and they had been starved for about three days before being fed the capsule, so that normal phosphorus metabolism might be hindered by a lack of some additional, essential nutrients. The data, however, show that incorporation of a dietary phosphate into bone tissue proceeds in the complete absence of dietary calcium, although, by far, most of the labeled phosphorus was recovered in the muscle tissue. The fish retained this phosphorus, in the absence of further feeding, very tenaciously, and did use a significant portion to form organic phosphorus compounds.

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## WESTERN FISH NUTRITION LABORATORY

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### HIGHLIGHTS

Quantitative protein requirements of sockeye salmon and rainbow trout were determined as 45 percent of diet in 10°C water, thus reinforcing high protein need for two more salmonids. Rate of protein synthesis in fish was reflected in four fold leucine aminopeptidase activity.

Chemistry and characteristics of blood clot fibers were studied here and abroad. Blood clot dissolving enzymes were assayed and results compared with activity in other animals.

Thiamin and pyridoxine status in fish was estimated with erythrocyte transketolase and erythrocyte transaminase enzymes systems.

New fractions of salmon endopeptidases disclosed unusual digestive enzymes in chinook salmon active in acid conditions of stomach and low caecum.

Iodide requirements of chinook salmon were estimated at one-half gram per ton and effect of low iodide overstressing kidney to increase susceptibility to disease was postulated.

Xanthine oxidase was found in salmon and trout, then used to measure status of animal and to detect nutritional imbalances. Application of enzyme to estimate biological value of diet protein was suggested.

An integrated Indispensable Amino Acid Index was proposed to improve and extend correlation of chemical score with biological value of protein in the diet.

Catfish blood amino acid patterns were accumulated and compared with feeding and fasting salmon. Comparative fasting amino acid patterns seemed poor estimates of actual indispensable amino acid requirements.



Nutritional score of dietary protein for fish was tested with improved PER and NPU techniques. Carbohydrate digestibility coefficient experiments were initiated to measure availability of materials in practical diets.

Salmon and trout thyroid function experiments were extended from young salmon and trout to migrating adults with and without thyroid.  $I^{125}$  turnover studies were conducted on thyroidectomized adult salmon and trout.

Water, copper and zinc concentrations showed adverse effect in embryonic development of salmon. Yolk fry showed greater sensitivity to copper ions than the eggs. Zinc toxicity in different waters was calculated for levels and time for LD<sub>50</sub>.

Adeno, trabecular and mixed carcinoma was observed in terminal samples of hepatoma I study. The list of carcinogens for trout was extended to include thioacetamide, aminotriazole and diethylstilbesterol; previous reports on other compounds were reinforced after all samples were reviewed.

Bioassay in hepatoma II study was completed at 20 months on test. Fish fed pellets, fat, and neutral lipid fraction, had tumors at each period and level tested.

Hepatoma III studies showed tumors in fish at six months fed crude aflatoxin, neutral lipid, total fat and beta subfraction. All died when fed heat polymerized fat for three weeks. Neutral lipid was fractionated into alpha, beta, gamma, delta and epsilon subfractions. Alpha was refractionated into five components and functional group tests on each performed. Beta was subfractionated into four sub-components and incorporated into hepatoma IV design.

Fish showed general absence of N-hydroxylase for 2-acetylaminofluorene but 3-OH, 5-OH and 7-OH AAF were found in the urine of fish given AAF.

LD<sub>50</sub> for aflatoxin in young trout was estimated at 3mg/kg/day (15 mg/kg total dose)

for the crude material. Quail tests were unsuccessful for aflatoxin but the quail were very sensitive to chlorinated hydrocarbons and organic solvent residues. Fluorescent spectrum and infrared spectrum of pure aflatoxin was completed to extend reliable physical characteristics of these mycotoxins.

Histopathology of hepatoma and other tissue anomalies from hepatoma I, II, and III studies was expanded with new rapid survey techniques. Studies with aflatoxin, polymerized fat and CHILLS factor showed typical toxicological response in liver, kidney, stomach, and musculature.

A new type of hemangiopericytomas or capillary hemangioma was found in pancreas of trout. Gonads of thirteen-year-old brook trout were degenerate or atrophied, but other tissues appeared normal.

The laboratory was privileged to send representatives to international meetings and conferences on status of fish husbandry research, hepatomagenesis and fish physiology, and to represent the Department at the Sixth International Congress of Nutrition and on an NIH Advisory Council Subcommittee on Carcinogenesis.

The new laboratory for fish physiology and lipid biochemistry was completed with proper ventilation and other equipment to handle large-scale solvent experiments. New tools and techniques increased rate of data and knowledge accumulation on nutrition, biochemistry, physiology and metabolism of salmonids.

## NUTRITION AND BIOCHEMISTRY

### Quantitative protein requirements of sockeye salmon and rainbow trout

The quantitative protein requirement techniques used to define the simulated whole egg protein requirement of chinook salmon at two or more water temperatures were adapted to measure the gross protein requirement of sockeye salmon and rainbow trout at an intermediate water temperature. A cooperative study with Purdue University was developed to determine

these protein requirements as part of a graduate thesis program. Simulated whole egg protein diets were formulated from casein and gelatin mixtures to which crystalline L-amino acids were added until the total amino acid pattern was identical with that of the corresponding analysis of whole egg protein. Isocaloric diets were made with gross protein contents at 25, 35, 40, 45, 50, 55 and 65 percent of simulated whole egg protein. Diets were maintained isocaloric by adjusting the white dextrin content of the ration, and all other dietary ingredients were maintained constant throughout the course of the experiment at that level normally used in the 50 percent protein-casein-gelatin control test diet. Plots of growth index versus total egg protein content of the diet of paired groups of young sockeye salmon and rainbow trout fed those diets showed classical inflection points at 45 percent of the whole egg protein pattern as the requirement for the two species under these environmental and experimental conditions. Almquist-type plots were constructed after each bi-weekly weigh period (Figures 1 and 2). All inflection points for individual plots consistently appeared at between 45 and 50 percent simulated whole egg protein.

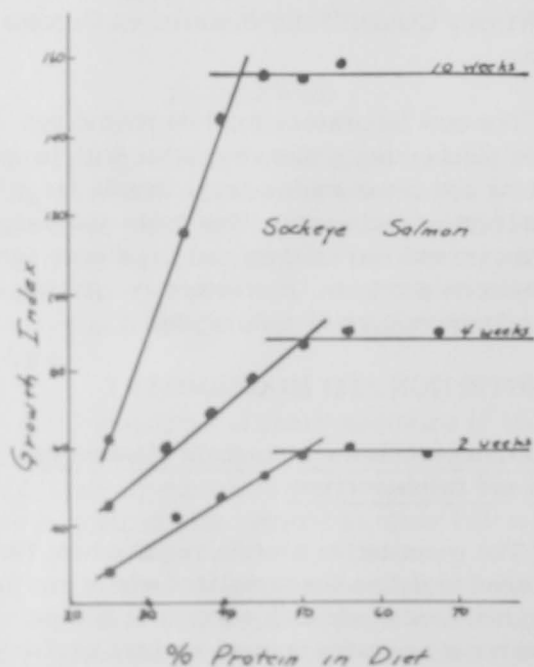


Figure 1:--Growth responses of young sockeye salmon fed simulated whole egg protein diets in 10° C. water

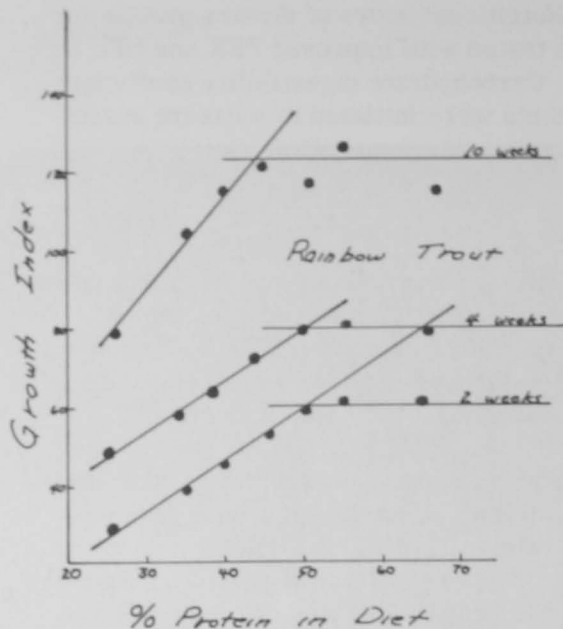


Figure 2:--Growth responses of young rainbow trout fed simulated whole egg protein diets in 10° C. water.

After 10 weeks of feeding, maximum growth index was obtained for those lots of fish fed 45 percent or more of protein in the diet. Mortality during the course of the 10-week feeding experiment was very low in both species tested. While terminal performance was consistently uniform at about 45 percent of protein after 10 weeks of feeding, during the first 6 weeks of feeding rainbow trout the inflection points appeared at about 50 percent of the gross dietary protein treatment, and then shifted in the last four weeks to the 45 percent of protein in the diet (see figures 1 and 2).

These experiments were not designed to measure the effects of varying protein intake at different fish sizes, and the effect of changing these protein intakes as the fish developed is speculative. Changes in gross dietary protein requirements have been observed in many other species examined closely under defined nutritional and experimental conditions as the animals develop in size and age. Postulates of this effect in fish have been incorporated into commercial diet manufacture without supporting experimental evidence and it is not surprising to uncover experimental data indicating that the gross dietary protein requirement does shift

from that needed by the very young rapidly growing animal toward a still high but less discrete and demanding requirement as the animal develops. Feeding tables have always reflected the size effect, the water temperature effect and may be redesigned to consider also gross dietary requirement protein effects.

Simulated whole egg protein was selected as the base protein in this series of experiments because of its high reference chemical score and its excellent biological value when tested with many other experimental animals. This protein has satisfied the nutritional score of many species and appears to be readily available and highly utilized by salmon and trout.

#### Leucine aminopeptidase activity

In preliminary experiments Dr. Julius Golubow of New York Hospital found very elevated leucine aminopeptidase activity in both tumor bearing and non-tumor bearing rainbow trout blood plasma. About 400 percent of normal mammalian activity was observed in only 7 samples assayed and one sample indicated that about 8 times the activity of normal human tissue was present. In efforts to establish whether this elevated activity may reflect the dynamic state of protein synthesis in a fish requiring nearly 50 percent of the food as gross protein, plasma preparations from the samples collected for the erythrocyte transketolase and erythrocyte transaminase activity studies were prepared for Dr. Golubow. In this cooperative study, fish having suspected different protein requirements were captured and blood plasma samples prepared for the leucine aminopeptidase activity tests from carp, sucker, shad, lamprey, rockfish, sculpin, dogfish, flounder, hake, sole, greenling, cod, steelhead trout, chinook, silver and sockeye salmon.

It is anticipated that Dr. Golubow will be able to accumulate some standard levels of leucine aminopeptidase activity in fishes and may from these preliminary studies accumulate enough evidence to design future experiments on the use of leucine aminopeptidase activity as an important clinical nutritional diagnostic test.

#### Salmon fibrinogen characterization

In cooperation with Dr. Jules Gladner of the National Institutes of Health and Professor Maurice Fontaine at the Museum of Natural History in Paris, a comparative biochemistry characterization of different fibrinogens from fishes and eels, was studied. Dr. Gladner, working with Professor Fontaine in Paris, assayed for amino acid sequences, fibrinogen and fibrin, stereo chemical configuration, molecular weight determinations and other physical-biochemical parameters to describe the structure, function and activity of this component in blood clotting mechanisms. Experimental animals selected for this study have been the chinook salmon, the lamprey, and the European eel. Lyophilized samples of sodium citrate preserved migrating male chinook salmon blood plasma have been prepared and were mailed to Dr. Gladner in October. From these studies we expect to accumulate information describing the physio-physical-biochemical characterization of chinook salmon and lamprey fibrinogen and learn more about its specific role and function in the mechanisms of fish blood clotting.

#### Chinook salmon fibrinolysin

In a cooperative study with Purdue University the specific activity of fibrinolysin in chinook salmon blood was investigated by Dr. Mertz and others cooperating with him. Oxalated migrating male chinook salmon blood plasma was prepared and shipped frozen to Purdue University for these studies. There, the specific activity of fibrinolysin, the blood clot dissolving enzyme, was assayed and the results compared with fibrinolysin activity in many other experimental animals. Specific substrates, environmental limits and their effect upon activity and exact physical-biochemical activities involved were studied. Comparative biochemical information should be assembled on the absence, or presence and activity of this enzyme which plays an important role in elimination of blood clots in active biological systems which may occur from stress, contusions, or other physiological shocks.

#### Thiamin and pyridoxine status estimate

Erythrocyte transketolase and erythrocyte transaminase activity were found to be

present in rainbow trout samples exposed to various nutritional treatments at this laboratory by Dr. Myron Brin from the New York Upstate Medical Center at Syracuse. Dr. Brin arrived at the laboratory in July and studied measurement of erythrocyte transketolase and erythrocyte transaminase specific activity, relating the values found to the specific nutritional status of thiamin and pyridoxine deficiencies in rainbow trout. After 14 weeks of feeding a thiamin deficient diet to larger rainbow trout, specific deficiency syndromes appeared and erythrocyte transketolase activity was reduced. In one fish the metabolic activity was reduced dramatically, the fish became edematous, floating on or near the surface with little or no muscular activity. Two hours after injection of an appropriate amount of co-carboxylase, the thiamin containing co-enzyme, the edema disappeared, the fish became upright and active and commenced feeding. Further studies of samples of hemolyzed red blood cells from these fishes given different nutritional treatments were prepared and were transported by Dr. Brin back to Syracuse where they are currently under assay. Samples of materials from rainbow trout fed the deficient diets at Hagerman water temperature showed a consistent reduction in activity, whereas other experiments at the Willard water temperatures were less definite.

In addition to the trout studies Dr. Brin has assayed for activity in lysed, washed erythrocytes from carp, sucker, shad, steelhead trout, chinook, silver, sockeye salmon, lamprey, three species of rockfish, sculpin, dogfish, flounder, sole, hake, greenling, ling cod, migrating steelhead trout and spawning chinook salmon of different sexes. From this survey of activity of these two enzymes present in the red blood cells of a number of fish tissues, standard values for resting enzyme activities and the specific response of this activity to thiamin and pyridoxine containing coenzymes will be used to determine and clinically evaluate nutritional status and response of various populations to dietary history.

#### Fractions of salmon caecal endopeptidases

The chromatographic study of the salmon endopeptidases has disclosed a number of frac-

tions which are practically unknowns. In relating them to the better known mammalian enzymes of apparently the same group it has been found that there are fish enzymes which resemble chromatographically and in general properties mammalian trypsin, alpha-chymotrypsin, and chymotrypsin B. However, some minor differences have been noted between these fish and mammalian enzymes and detailed studies could easily reveal more differences just as is now finally being recognized for long-known mammalian enzymes (e.g. bovine vs. porcine trypsin). In addition, several fish enzymes have been noted which appear to have the general function of endopeptidases but show more pronounced differences in other properties initially evidenced by chromatographic behavior. The latter are the ones which we have selected to study first in more detail. It must be kept in mind that they are "unusual" or "extraordinary" only in respect to what is known about mammalian enzymes; they appear normal at least to salmonids.

Perhaps the most interesting of the unusual endopeptidases is "chromatographic Fraction IV" (formerly sometimes referred to as "salmotrypsin IV") which appears to have the general function of trypsin. It comes off last from a column of DEAE-cellulose in our regular elution procedure whereas the other trypsin-like fish activity and the mammalian trypsin are not even held on the column. More refined chromatographic treatment has shown this fraction to be composed of two rather diffuse subfractions--IV<sub>a</sub> and IV<sub>b</sub> (1962 Annual Report). A small quantity of this fraction has been prepared in a relatively pure form by batch isolation procedures making use of a previously reported but unexplained phenomenon of its initial partial insolubility at pH 4.5 along with all the other known purification steps. A continuing study is in progress to improve the isolation scheme which was too laborious and at times too arbitrary for repetition to the desired quantity of pure material. Electrophoretic studies of the purified preparation showed two main components (Figure 3) and each tested positive for TAME-hydrolyzing activity thus confirming the chromatographic findings. Mobilities calculated from the electrophoretic data at six pH's between 4 and 6 were plotted (Figure 4) to show isoelectric points of 5.12 and 4.56 for Fractions



IV<sub>a</sub> and IV<sub>b</sub>, respectively. This accurately defines the previously reported low (acid) isoelectric points of these enzymes, as contrasted to the highly alkaline isoelectric point of about 10 for trypsin.

The other fish proteinase under further study is a chromatographic fraction eluted from a CM-cellulose column after the elution of the fraction which appears to be more closely related to mammalian alpha-chymotrypsin. (This chromatography was conducted on the components of the break-through peak of the DEAE cellulose chromatography). Both fractions were ATEE-hydrolyzing and fit the general classification of a chymotrypsin. The possibility existed that the fraction of delayed elution although different from alpha-chymotrypsin in that respect, was chromatographing like one of the other known mammalian chymotrypsins derivable from chymotrypsinogen A. This was shown to be not the case by also chromatographing under like conditions commercially available beta, gamma, and epsilon chymotrypsins which eluted like a-chymotrypsin.

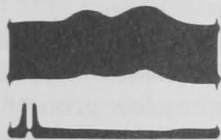


Figure 3:--Electrophoretic pattern of purified Fraction IV of salmon endopeptidases. Descending boundary at pH 6.0.

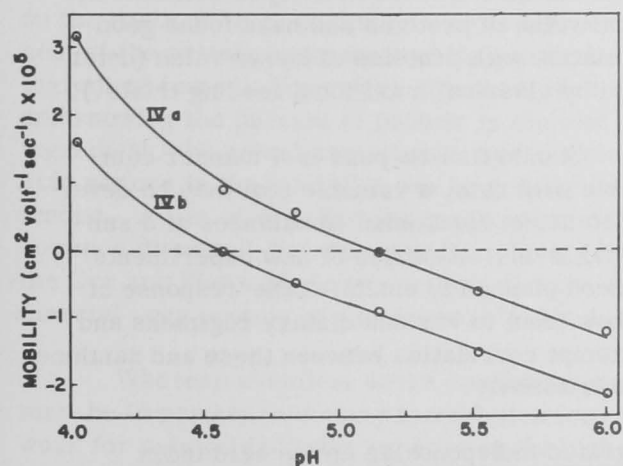


Figure 4:--Electrophoretic mobility of salmon endopeptidase Fractions IV<sub>a</sub> and IV<sub>b</sub> as a function of pH.

### Iodide requirements of chinook salmon

Iodide requirements of chinook salmon were determined by feeding groups of fish a low iodide test ration to which was added sodium iodide to given final iodide concentrations of 0.1, 0.6, 1.1, 5.1, and 10.1 micrograms of iodide per gram dry diet. The iodide content of the water in which the fish were raised varied from 0.1 to 0.3 micrograms of iodine per liter during the experimental period. Based on maximum accumulation of iodine in the thyroid the requirement of the fingerlings was approximately 0.6  $\mu\text{gI}^-$  per g dry diet during the first 24 weeks of the experiment. For advanced parr at age 15 months the requirement approximately doubled. Figure 5 illustrates these conclusions. No significant difference in weight gain, feed efficiency or mortality was found between groups during the first 24 weeks.

During the last 3 months of the extended feeding period a persistent mortality occurred in the fish fed the lowest iodide level which resulted in an 82 percent loss in this group by the end of the feeding trial. During the last 2 months the groups fed the next lowest levels of iodide suffered a 24 and 22 percent loss while the group fed the highest two levels had only 5 and 4 percent loss respectively. At the termination of the feeding trial, evidence of kidney disease was found in all groups of fish except those which received the highest level of dietary iodide. In view of the known thyroid function in osmotic regulation, it was postulated that a thyroid deficiency might result in an overstressed kidney which would then be more susceptible to the KD organism.

### Dietary calcium requirements of salmon

The effect of environmental calcium on the dietary calcium requirements of chinook fingerlings was investigated. Replicate lots of fish were fed added increments of calcium gluconate to yield a basal ration with 5 levels of calcium (.125, .25, .50, .75, and 100 percent of basal). Duplicate groups were raised in untreated water (containing 3.5 ppm Ca) and in water to which an additional 20 ppm Ca was added. Added calcium was maintained in the water supply by metering a calcium chloride



concentrate into the line supplying water to the troughs. Although there was evidence of some growth retardation at higher calcium levels which was accentuated by calcium enrichment of the water, the experimental results were blurred by overcrowding which tended to limit growth toward the latter part of the experiment. The experiments will be repeated in 1964.

### Xanthine oxidase

Xanthine dehydrogenase activity was measured in the livers of rainbow trout and chinook salmon. The nature of this enzyme appeared to act as a dehydrogenase rather than as the oxidase found in mammals. A similar mode of action was reported with avian species, which also contain nucleated red blood cells. The criterion for classification as a dehydrogenase was the greatly reduced activity found in the absence of methylene blue. In respiring liver homogenates methylene blue was reported to act as a hydrogen acceptor for the enzyme. Since methylene blue is auto-oxidizable, the activity of the enzyme can be translated into an increased oxygen consumption, (Remy et al. *J. Biol. Chem.* 193: 649-657 1951). Respiring homogenates of rainbow trout liver in the presence of xanthine as hypo-xanthine reacted in a similar manner when methylene blue was added to the flask. In contrast with certain mammals, activity in the trout appeared to be limited to the liver. Kidney, muscle, plasma and erythrocyte preparations were negative for the enzyme.

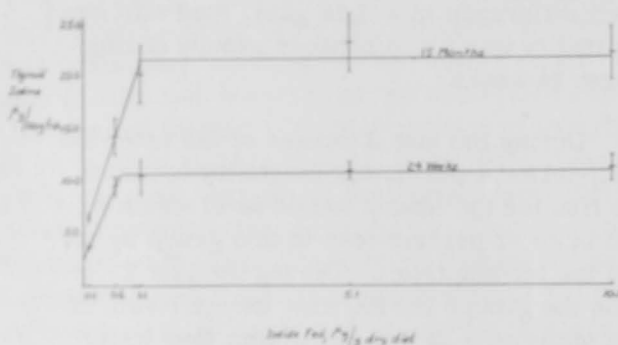


Figure 5:--Thyroidal iodine storage in chinook salmon fingerlings and parr fed diets containing varied levels of iodide. Cross bar represents the mean value of 10 individual fish, the extent of the vertical line represents the standard error of estimate.

### Chinook salmon requirement for iron and copper

The requirement of chinook salmon for dietary iron and copper was investigated in a 24-week feeding trial. The fish were fed an iron and copper "deficient" basal ration to which was added salts of the two elements to yield diets containing all combinations of 4 levels of each element. Results of the feeding trial indicated that the lowest levels of each element fed was sufficient to prevent the recurrence of anemia or to cause significant changes in growth. It will thus be necessary to reduce the iron and copper content of the basal test ration before the requirements for these elements can be defined.

In other animals this enzyme system has been correlated with a variety of nutritional conditions. For example, low protein diets have caused a profound reduction in liver enzyme level. Certain vitamin deficiencies, notably pyridoxine and B<sub>12</sub> have been correlated with low levels of activity. Litevach and other authors have employed xanthine oxidase activity to estimate biological value of proteins and have found good correlation with proteins of known value (determined by classical nutritional feeding trials).

Should fish respond in a manner comparable with rats, a valuable tool may be developed to detect nutritional imbalances at a sub-clinical level. A series of new experiments has been planned to establish the response of rainbow trout to various dietary regimens and to attempt correlation between these and xanthine oxidase activity.

### Integrated indispensable amino acid index

Amino acid content and chemical scores of fish diets have been a major area of research in the laboratory. The purchase of the automatic

amino acid analyzer has led to significant progress in accumulating chemical scores and amino acid contents of a variety of dietary ingredients. In the past year a series of diets and individual diet components have been analyzed. The major objective of this program has been to compile a catalogue of diet components in which the complete amino acid pattern is carefully defined. Over 30 samples of commercial, hatchery, and experimental diets plus the various dietary ingredients have been assayed. To make the accumulated information of greater utility, chemical scores have been calculated on a number of these ingredients using the classical method of Oser. The values listed for whole egg protein are those of Orr and Watt and were determined by microbiological assay techniques. To establish a better correlation between these values and other literature data, a sample of commercially available whole egg was included for comparison purposes. Chemical scoring is a method of predicting the biological value on the basis of the level of indispensable amino acids present in the particular protein tested. This technique merely assembles the specific indispensable amino acid contribution from each of these acids and relates this pattern with that present in whole egg protein. Using this technique one can then determine the first limiting amino acid present in a particular test protein and at what point inclusion of a particular dietary protein ingredient would be predicted in the diet formulation. For comparison purposes, the chemical scores are normally calculated on the basis of 16 g. of nitrogen or at 100 percent of the protein component of the particular ingredient tested. From this standard value and knowing the percent of protein in the test ingredient, the actual contribution to the amino acid pattern in the total diet can be readily extended. A list of the chemical score of some common diets and dietary ingredients using both the Orr and Watts whole egg protein and the commercial egg protein can be found in Table 1.

Whereas chemical score considerations may be important in dietary formulation for work for salmon and trout because of the high protein requirement of these species, even more directly applicable information can be obtained from careful consideration of an integrated indispensable amino acid index. This essential

amino acid index can be defined as the geometric means of the indispensable amino acid pattern in any test protein with respect to individual amounts of each in a reference whole egg protein.

$$I\text{IAAI} = 100 \sqrt[n]{\frac{AA_1^P}{AA_1^R} \times \frac{AA_2^P}{AA_2^R} \times \dots \times \frac{AA_n^P}{AA_n^R}} \quad \begin{matrix} 1 \\ n \\ 0 \end{matrix}$$

Where AAP = gm of AA per gm N of test protein; AA<sup>R</sup> = gm of AA per gm N of reference protein, and n = number of indispensable amino acids considered.

The integrated indispensable amino acid index (IIAAI) can be mathematically described as the nth root of the product of the ratios of the specific required indispensable amino acids content with respect to the test protein divided by the content of the same amino acids in the reference protein. Here p is test protein, r is reference protein and n is the number of indispensable amino acids considered for the species. Individual amino acid ratios are logically limited between bounds of 0 and 1. This particular calculation avoids the necessity of applying conventional protein factors such as 6.25, 5.7, 6.0 etc., used to convert analytically determined nitrogen into protein content. Mathematical treatment can be improved if data for cystine and tyrosine are considered with the individual segments for methionine and phenylalanine, respectively. Application of the IIAAI to fish diet formulation can now be used with a greater degree of confidence since most of the analytical data listing the amino contents of a number of dietary sources have all been referred to whole egg protein. In repetitive experiments it has been demonstrated that simulated whole egg protein patterns were also readily utilized by salmon and trout to satisfy the indispensable amino acid requirement of these test species.

An obvious limitation of the application of either chemical score or Indispensable Amino Acid Index to final diet formulation must be a realization that chemical analysis of the amino acid content of any ingredient must be coupled with a digestibility and utilization coefficient before the amount of specific amino acid present in the diet can be correlated with what the individual fish actually uses from what it eats.

Table 1:--Chemical score of diet protein

<u>Dietary Ingredients</u>	<u>Orr &amp; Watts Whole Egg</u>	<u>1st Limiting</u>	<u>Commercial Whole Egg</u>	<u>1st Limiting Amino Acid</u>
Whole Egg-Orr & Watts	100	None	99	methionine
Whole Egg-Commercial	90	tryptophan	100	none
Salmon Egg	85	tryptophan	91	tryptophan
Casein	82	arginine	88	arginine
Past. Salmon Carcass aps	78	tryptophan	86	tryptophan
Autolyzed Salmon Carcass aa	78	tryptophan	86	tryptophan
Herring Meal	76	tryptophan	84	tryptophan
Commercial Diet #1 acr	76	tryptophan	84	methionine
Commercial Diet #2 acs	76	tryptophan	83	methionine
Skim Milk	76	tryptophan	83	tryptophan
Drackett	75	methionine	82	methionine
Salmon Viscera	74	tryptophan	82	tryptophan
Fishes Wishes	74	tryptophan	82	tryptophan
Experimental Diet	73	tryptophan	80	tryptophan
Turbot	72	tryptophan	79	tryptophan
Soybean Meal	71	methionine	76	methionine
Commercial Diet #3 am	71	tryptophan	79	methionine
Commercial Diet #4 adf	69	tryptophan	76	methionine
O.M.P.	69	tryptophan	76	methionine
McNenny	68	tryptophan	75	tryptophan
Rangens	68	tryptophan	75	tryptophan
Salmon Meal	67	isoleucine	74	valine
Tuna Viscera	66	tryptophan	73	tryptophan
Sesame	66	lysine	73	16sine
Brewers Yeast	63	methionine	70	methionine
Wheat Germ Meal	62	tryptophan	68	methionine
Distillers Solubles	59	tryptophan	65	tryptophan
Cottonseed Meal	59	tryptophan	65	tryptophan
Wheat Middlings	56	isoleucine	62	methionine
Shrimp Meal	50	tryptophan	56	tryptophan
Crab Solubles	44	tryptophan	48	tryptophan

### Catfish blood amino acid patterns

Studies of the free amino acid pattern in the blood of three species of catfish along with values obtained from chinook salmon can be reviewed in Table 2. Catfish samples were prepared at the Southeastern Fish Cultural Laboratory at Marion, Alabama; salmon were taken from the high seas and various stages in the migration up the Columbia River. A list of the specific amino acids found in the samples should stimulate comment and interest in this technique for estimation of the specific amino acids requirements from the blood levels found after different periods of inanition. As can be seen from an examination of the amino acids listed

the trough at which the anabolic processes exactly balance catabolic processes cannot be discretely plotted. Interpretation of these studies must therefore remain dependent upon more careful studies of the responses of individual fish and on the levels found in one group of experimental fishes with more positive experimental control over the various nutrients in question.

### Comparative fasting amino acid patterns

An extensive roundtable discussion was organized at the Sixth International Congress of Nutrition in Edinburgh, to consider the significance of fasting blood amino acid patterns as a technique for estimating the indispensable amino

Table 2:-- Plasma amino acid patterns of fish

Amino Acid	Chinook Salmon				Fasting Catfish			Catfish		
	Ocean Feeding	24 hrs Fasting	Spawning Male	Spawning Female	48 hrs	120 hrs	216 hrs	Blue	Channel	Flathead
Phosphoserine			0.5	0.4	2.3	2.7	2.8			
Glycerophosphoethanolamine	2.6	3.6	2.8	2.9	4.3	6.7	5.9	5.3	5.2	3.3
Phosphoethanolamine	24	11	16	18	2.7	2.0	6.0	2.9	5.6	2.9
Taurine	81	61	199	201	63	63	98	30	95	36
Urea	62	150	55	19	14	13	21	18	22	19
Methionine										
Sulfoxides	6.2	2.4	3.3	2.7	3.0	3.9	4.9	0.93	3.5	1.1
Hydroxyproline	7.6				5.8	5.9	6.2	4.1	2.9	14
Aspartic Acid	3.8	3.4	4.7	4.9	1.9	2.2	10	0.84	1.2	0.82
Threonine	58	17	37	32	15	18	26	11	10	13
Serine	28	8.9	397	108	4.5	10	9.5	7.0	4.6	8.6
Asparagine										
Glutamine	30	36	34	29	12	14	7.6	14	12	9.3
Sarcosine					--	--	1.7	--	--	1.3
Proline	21	11	18	14	7.4	6.0	12	5.4	7.9	6.5
Glutamic Acid	27	18	28	28	4.8	5.2	18	7.8	13	7.7
Citrulline	11	7.4	7.6	8.3	--	--	--	--	--	--
Glycine	42	35	20	52	8.9	11	23	6.1	3.1	4.8
Alanine	146	54	107	108	6.8	7.6	14	7.5	9.7	9.1
$\alpha$ -Amino-n-butyric Acid	5.0	4.1	4.7	4.5	1.1	0.93	1.0	0.87		0.85
Valine	75	90	101	62	17	19	27	22	13	13
Cystine			3.6	62	2.4	3.1	4.6	4.8	3.2	2.4
Cystathionine	2.3	1.3	62	73	0.5	0.4	0.6	0.4	0.4	0.3
Methionine	13	3.2	19	12	0.3	0.4	0.3	0.4	0.4	0.4
Methionine + Methionine Sulfoxides	19	5.6	22	14	3.3	4.3	5.2	1.3	3.9	1.5
Isoleucine	42	47	50	32	15	17	22	24	13	12
Leucine	70	82	87	50	10	16	20	29	19	23
Tyrosine	28	13	16	16	4.1	5.2	7.2	14	6.3	8.0
Phenylalanine	24	12	24	19	7.3	8.2	11	9.3	5.7	13
$\beta$ -Alanine	1.8	1.1	10	11	2.9	6.7	4.1	0.9	1.4	--
$\beta$ -Aminoisobutyric Acid			2.2	1.3	0.7	0.7	0.4	--	--	--
Galactosamine					5.6	5.5	5.4	1.4	3.1	--
Hydroxylysine	0.8	0.3	0.5	0.7	0.7	1.1	1.8	0.6	0.5	--
$\gamma$ -Aminobutyric Acid	4.5	6.3	11	12	0.8	0.41	0.63	2.0	1.7	1.3
Ornithine	3.7	2.2	10	5.8	7.6	12	25	20	2.8	4.3
Ethanolamine	1.7	2.3	0.9	1.1	2.0	2.3	2.7	0.8	1.2	0.8
Ammonia	22	13	18	15	7.3	7.9	21	8.8	9.1	6.0
Lysine	51	10	108	88	16	24	39	12	11	10
1-Methylhistidine	1.2	1.0	139	30	5.6	6.1	7.8	0.9	--	--
Histidine	23	18	13	10	3.3	3.9	6.4	3.1	3.0	6.0
3-Methylhistidine			1.7	0.42	--	--	--	--	--	--
Tryptophan		0.5	22	6.6	--	--	--	4.0	--	--
Arginine	34	3.0	24	23	5.4	9.1	9.8	5.2	2.5	2.1

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Commercial Diet #4 adf	69	tryptophan	76	methionine
O.M.P.	69	tryptophan	76	methionine
McNenny	68	tryptophan	75	tryptophan
Rangens	68	tryptophan	75	tryptophan
Salmon Meal	67	isoleucine	74	valine
Tuna Viscera	66	tryptophan	73	tryptophan
Sesame	66	lysine	73	l6sine
Brewers Yeast	63	methionine	70	methionine
Wheat Germ Meal	62	tryptophan	68	methionine
Distillers Solubles	59	tryptophan	65	tryptophan
Cottonseed Meal	59	tryptophan	65	tryptophan
Wheat Middlings	56	isoleucine	62	methionine
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Phosphoserine			0.5	0.4	2.3	2.7	2.8			
Glycerophosphoethanolamine	2.6	3.6	2.8	2.9	4.3	6.7	5.9	5.3	5.2	3.3
Phosphoethanolamine	24	11	16	18	2.7	2.0	6.0	2.9	5.6	2.9
Taurine	81	61	199	201	63	63	98	30	95	36
Urea	62	150	55	19	14	13	21	18	22	19
Methionine										
Sulfoxides	6.2	2.4	3.3	2.7	3.0	3.9	4.9	0.93	3.5	1.1
Hydroxyproline	7.6				5.8	5.9	6.2	4.1	2.9	14
Aspartic Acid	3.8	3.4	4.7	4.9	1.9	2.2	10	0.84	1.2	0.82
Threonine	58	17	37	32	15	18	26	11	10	13
Serine	28	8.9	397	108	4.5	10	9.5	7.0	4.6	8.6
Asparagine										
Glutamine	30	36	34	29	12	14	7.6	14	12	9.3
Sarcosine					--	--	1.7	--	--	1.3
Proline	21	11	18	14	7.4	6.0	12	5.4	7.9	6.5
Glutamic Acid	27	18	28	28	4.8	5.2	18	7.8	13	7.7
Citrulline	11	7.4	7.6	8.3	--	--	--	--	--	--
Glycine	42	35	20	52	8.9	11	23	6.1	3.1	4.8
Alanine	146	54	107	108	6.8	7.6	14	7.5	9.7	9.1
$\alpha$ -Amino-n-butyric Acid	5.0	4.1	4.7	4.5	1.1	0.93	1.0	0.87		0.85
Valine	75	90	101	62	17	19	27	22	13	13
Cystine			3.6	62	2.4	3.1	4.6	4.8	3.2	2.4
Cystathionine	2.3	1.3	62	73	0.5	0.4	0.6	0.4	0.4	0.3
Methionine	13	3.2	19	12	0.3	0.4	0.3	0.4	0.4	0.4
Methionine + Methionine Sulfoxides	19	5.6	22	14	3.3	4.3	5.2	1.3	3.9	1.5
Isoleucine	42	47	50	32	15	17	22	24	13	12
Leucine	70	82	87	50	10	16	20	29	19	23
Tyrosine	28	13	16	16	4.1	5.2	7.2	14	6.3	8.0
Phenylalanine	24	12	24	19	7.3	8.2	11	9.3	5.7	13
$\beta$ -Alanine	1.8	1.1	10	11	2.9	6.7	4.1	0.9	1.4	--
$\beta$ -Aminoisobutyric Acid			2.2	1.3	0.7	0.7	0.4	--	--	--
Galactosamine					5.6	5.5	5.4	1.4	3.1	--
Hydroxylysine	0.8	0.3	0.5	0.7	0.7	1.1	1.8	0.6	0.5	--
$\gamma$ -Aminobutyric Acid	4.5	6.3	11	12	0.8	0.41	0.63	2.0	1.7	1.3
Ornithine	3.7	2.2	10	5.8	7.6	12	25	20	2.8	4.3
Ethanolamine	1.7	2.3	0.9	1.1	2.0	2.3	2.7	0.8	1.2	0.8
Ammonia	22	13	18	15	7.3	7.9	21	8.8	9.1	6.0
Lysine	51	10	108	88	16	24	39	12	11	10
1-Methylhistidine	1.2	1.0	139	30	5.6	6.1	7.8	0.9	--	--
Histidine	23	18	13	10	3.3	3.9	6.4	3.1	3.0	6.0
3-Methylhistidine			1.7	0.42	--	--	--	--	--	--
Tryptophan		0.5	22	6.6	--	--	--	4.0	--	--
Arginine	34	3.0	24	23	5.4	9.1	9.8	5.2	2.5	2.1

acid (IAA) requirements of different animals. Both the Jarowski technique of calculating minimum individual amino acid requirements from measurements of plasma volume (liters per kilogram) times body weight (kilograms) times the fasting amino acid level (milligrams per liter) times a corrective factor for individual species and weight was discussed and compared with the Longnecker-Hause method of estimating the requirements from the amino acid pool value minus the fasting value divided by the specific amino acid requirements times 100. Each of these standard proposed methods as well as more elegant extensions of each individual method, assume presence of a trough in the fasting blood free amino acid pattern at which time the contribution from the dietary protein is exactly balanced by the essential catabolic processes in the body when food supplies of amino acids are no longer being ingested. The basic assumption that this trough would appear at a common time for each of the 10 indispensable amino acids was severely challenged and considered presumptive because of the known metabolic pathways of the various amino acids and the multiple uses that each has in body metabolism. Therefore, it was suggested that more work was needed to measure the particular point at which each of the IAA present in a wide variety of protein sources might disappear from the circulating blood, and to establish whether a mean trough did exist or whether individual troughs did appear for each of the 10 amino acids.

Using a lysine ratio of 1.0, comparative fasting patterns were developed for the circulating free amino acids in a number of animals and were compared with a normal and phenylketonuric human. As seen from table 3, the indispensable amino acid and non-essential amino acid total pattern ratios between the salmon, catfish and pig were comparable. When lysine values were adjusted to 1.0, the catfish, pig, dog, and human compared favorably whereas the 20-pound salmon fasted for 24 hours had a very elevated valine, isoleucine and leucine plasma amino acid content.

Further work is contemplated on younger sockeye salmon and rainbow trout which have been fed various amounts of simulated whole egg

protein pattern in the diet. Blood plasma preparations from the terminal samples of these protein requirement studies have been prepared and will be analyzed on the amino acid analyzer at Purdue University by Mr. Bates during the fall and spring semesters. From these data one should be able to resolve (a) presence and time appearance of trough for each indispensable amino acid; (b) changes in fasting amino acid pattern of actively growing young salmon and trout, and (c) applicability of the Jarowski or Longnecker-Hause techniques for estimation of minimum amino acid quantitative requirements from blood samples of various fishes starved standard time periods.

#### Nutritive value of proteins

The high protein requirement of salmonid fishes (2 to 4 times that of rats and chickens) is by now, well documented. The economic impact of rising dietary protein costs might be considered, therefore, to be 2 to 4 times as great as in other areas of animal husbandry. The above, when accompanied by the recent rapid strides made in definition of basic nutritional requirements of fish, particularly salmonids, serves to emphasize that the need for a reliable, reproducible and uncomplicated method for measuring relative nutritive quality of dietary ingredients is of similar magnitude.

Fundamental experimental techniques for measuring nutritive value have been applied to a variety of animal husbandries, including fish. Nitrogen balance studies e.g. have been successfully applied to fish by Tunison and co-workers (Cortland Hatchery Reports 1940-43). Probably the most frequently employed parameter for measuring biologic value, however, is simple weight gain per unit of protein fed or Protein Efficiency Ratio. Good correlation has been found between PER chemical score and biologic values obtained with nitrogen balance techniques. Bender (Brit. J. Nutr. 10: 135 1956) observed that PER varied with food intake and to circumvent this variable factor calculated net protein utilization (NPU) from the carcass nitrogen of animals receiving the test protein and a non-protein diet respectively. Body nitrogen of the test animal minus body nitrogen of the non-protein group, divided by nitrogen intake of test protein,

Table 3:--Comparative Fasting Patterns

HOURS →	24	24	48	16-18	14	14	16-18
	20 lb. Salmon	25 lb. Pig	20 lb. Catfish	Human Adult	Child PKU	Child PKU + L	Adult Dog
Lysine	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Threonine	1.7	0.8	0.9	0.6	0.4	0.5	1.5
Valine	9.0	1.0	0.8	1.2	1.0	1.0	1.0
Cystine	--	0.4	0.2	0.6	0.5	0.6	0.4
Methionine	0.3	0.2	0.2	--	0.2	0.2	0.4
Isoleucine	4.7	0.7	0.9	0.46	0.25	0.23	0.5
Leucine	8.2	0.7	0.7	0.8	0.5	0.5	0.9
Phenylalanine	1.2	0.5	0.3		17.7	1.4	
Tyrosine	1.3	0.4	0.2	0.9	0.2	0.2	0.7
Arginine	0.3	0.7	0.4	--	0.5	0.3	1.1
Histidine	1.8	0.6	0.2	--	0.7	0.4	0.4
Tryptophan	0.05	0.1	--	0.5	0.2	0.1	0.4
EAA	208	202	98.7	--	252	142	--
NEAA	130	183	37.5	--	38	90	--
Total	338	385	136.2	--	290	232	--
E/N Ratio	1.6	1.1	2.6	--	6.6	1.6	--
Lysine(γ/ml)	28	10	17	25	11	22	--

equals NPU. This value was found to be independent of food intake; afforded a measure of maintenance requirement and correlated well with established biologic values.

The major objective of the feeding trials conducted this year was to test the practicability of PER as a measure of protein quality. A non-protein was also included to permit calculation of NPU. Two levels of casein (ad libitum and 2/3 the ad libitum level) were fed to determine the effect of intake on PER and NPU. A level of 20 percent protein (Nx6.25) was maintained in all diets, except as noted in Table 4. This level was selected on the basis of previous results which indicated maximum PER (but not maximum gain) to be obtained at approximately 20 percent of protein in the diet. Extreme variation in physical properties of diets precluded higher levels of protein, while reduced weight gains argued against levels lower than 20 percent. Details of feeding, techniques of handling the diet preparation have been described in previous reports. The protein source replaced an equivalent weight of starch and high fat containing materials which were defatted by conventional procedures. The results of the trials are summarized in tables 5 and 6.

In experiment 1 maximum PER and NPU were obtained with casein fed at restricted levels. Casein, fish meal, and beef liver, produced comparable protein efficiencies and NPU's; but weight gain and feed consumption with the casein diet were reduced. Wheat germ, dried corn, distiller's solubles and dried brewer's yeast produced lowest gains, lower PER and NPU. Cottonseed meal proved more acceptable in this trial as weight gain, feed consumption and PER were increased over previous trials. Dried whole egg solids, defatted by extraction for 24 hours, were fed for comparison with values found in rat experiments. It was of interest that, although consumption of the dried egg diet exceeded the plant proteins, lower PER values were obtained. It is believed the method of extraction followed here may have influenced the results since subsequent trials produced higher PER values with the same lot of material extracted by the Bloor technique.

The results of Experiment 2 are summarized in Table 6. Maximum PER was again obtained with casein, fish meal, beef liver and defatted salmon eggs (Bloor technique). Defatted whole egg produced PER higher than previously while values obtained with cottonseed meal,

Table 4:--Composition of diets experiments 1 and 2

Diet	Non-Prot.	Casein	Cotton Seed Meal	Fish Meal	Wheat Germ	Brewer's Yeast	Beef Liver	Defatted Whole Egg	Defatted Salmon Egg	Dist. Sols.
Basal Ration (1)	50.0 (1)	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Protein	-	22.5	46.6	26.7	50.0	42.4	104.0	25.1	26.1	50.0
Starch	25.0	27.5	4.4	23.3	-	7.6	30.0	24.9	23.9	-
a-Cell.	25.0	-	-	-	-	-	-	-	-	-
H <sub>2</sub> O	60.0	60.0	60.0	60.0	60.0	60.0	-	67.5	67.5	60.0
% Prot. (N X 6.5)	0.0	20.0	20.0	14.6	20.0	20.0	20.0	20.0	20.0	12.75

## COMPOSITION OF BASAL RATION (1)

## INGREDIENTS

Dextrin	25.0
Vitamin Supplement	9.0
Mineral Supplement	4.0
Corn oil	8.0
Cod Liver Oil	2.0
Carboxymethyl cellulose	2.0
	<u>50.0</u>

## VITAMIN SUPPLEMENT

To 8.0 gms. -cellulose are added (in mgs.):

Thiamine HCl	5.0
Riboflavin	20.0
Pyridoxine HCl	5.0
Choline Cl	500.0
Nicotinic Acid	75.0
Ca panthothenate	50.0
Inositol	200.0
Biotin	0.5
Folic Acid	1.5
Ascorbic Acid	100.0
B <sub>12</sub>	0.01
-Tocopherol( acetate)	40.0

## MINERALS SUPPLEMENT

To 100 gms. USP XIII Salt Mixture No. 2 are added (in mgs.):

AlCl <sub>3</sub>	15
ZnSO <sub>4</sub>	300
CuCl	10
MnSO <sub>4</sub>	80
KI	15
CoCl <sub>2</sub>	100

particularly with one lot of fish were lower. Wheat germ and brewer's yeast again showed lowest protein efficiencies. Net protein utilization data were in general repeated in the second experiment. The casein diets were utilized most efficiently followed by fish meal, defatted salmon eggs, beef liver, cottonseed meal, brewer's yeast and wheat germ, in that order. Maximum weight gain and feed consumption were achieved with fish meal and beef liver.

Protein efficiency ration as a measure of protein quality for rats is subject to at least 3 major criticisms: (1) weight gain does not necessarily reflect true body composition; (2) no allowance is made for maintenance requirements; and (3) PER has been shown to vary with intake. The first criticism holds true for fish from an examination of the proximate analysis data in Table 7. Soybean meal was not included because the fish refused to eat the diet. Percent fat ranged from 26.7 percent for casein to a high of 37.2 percent with beef liver. This explained the higher

NPU obtained with casein while PER values similar to beef liver and fish meal were recorded.

The second and third criticisms of PER, i. e. (2) no allowance is made for maintenance and (3) that values obtained vary with intake, may not be valid for fish under the test conditions described. It may be argued that the ability of the test protein to meet the maintenance requirement of the animal is expressed, if indirectly, by PER since growth can occur only after the maintenance requirement is met. Furthermore the amount of protein required to maintain the animal in nitrogen equilibrium is a function of the nutritive quality of the protein. In the experiments reported here variation of protein intake did not produce consistent differences in PER when a 20 percent casein diet was fed. Higher NPU values, however, were obtained with the restricted level of intake of casein which varies with Bender's observation that NPU (with rats) is independent of intake.

Table 5:-- Summary results

EXPERIMENT #1										
DIET PROTEIN	NUMBER FISH	BEGIN WEIGHT	FINAL WEIGHT	FOOD FED	PROTEIN FED	WEIGHT GAIN	PER	NPU		
Casein 'ad lib'	200	185.2	359.4	435.2	54.4	174.2	(3.95)*	3.20	(0.22)	49.8
	200	179.3	344.0	413.0	51.6	164.7		3.19		48.7 (1.57)
Casein 2/3 Intake	200	176.6	309.5	289.0	36.1	132.9	(8.96)	3.68	(5.36)	57.7
	200	178.5	295.7	275.2	34.4	117.2		3.41		55.4 (0.99)
Fish Meal	200	178.2	389.2	526.3	65.8	211.0	(6.79)	3.21	(0.44)	47.6
	200	176.4	408.7	583.4	72.9	232.0		3.19		46.4 (1.81)
Cottonseed Meal	200	177.4	255.5	289.9	36.2	78.1	(4.77)	2.16	(1.50)	30.7
	200	177.4	250.4	283.7	35.5	73.0		2.06		29.7 (0.74)
Wheat Germ	200	181.5	221.8	312.0	28.5	40.3	(2.89)	1.42	(0.98)	29.9
	200	180.0	222.0	319.2	29.1	42.0		1.44		29.4 (1.20)
Beef Liver	200	179.6	381.5	580.4	63.1	202.3	(5.78)	3.21	(5.17)	48.3
	200	188.0	373.0	571.8	62.1	184.9		2.98		47.3 (1.49)
Brewer's Yeast	200	182.8	242.5	337.6	42.2	59.7	(5.16)	1.41	(2.04)	24.4
	200	182.5	238.0	325.1	40.6	55.5		1.37		24.0 (1.16)
Defatted Whole Egg	200	187.7	242.3	355.6	44.4	54.6	(4.02)	1.23	(4.94)	23.1
	200	181.5	239.3	350.7	43.8	57.8		1.32		23.6 (1.52)
Distillers Solubles	200	185.0	213.6	264.7	21.9	28.6	(13.45)	1.31	(6.20)	---
	200	178.4	213.0	291.4	24.1	34.6		1.43		---- **
Soybean Meal	200	180.8	130.1	---	---	---	---	---	---	---
	200	178.0	149.7	***	---	---		---		---
Non-Protein	200	181.7	162.3	291.7	---	-21.4	---	---	---	---
	200	182.9	161.6	300.0	---	-21.8		---		---
	200	177.8	159.0	287.0	---	-18.8 (7.85)		---		---

\* Coefficient variation between lots

\*\* NPU Not calculated-Experiment continued

\*\*\* Fish refused diet.

Table 6:--Summary Results

EXPERIMENT #2										
DIET PROTEIN	NUMBER FISH	BEGIN WEIGHT	FINAL WEIGHT	FOOD FED	PROTEIN FED	WEIGHT GAIN	PER	NPU		
Casein	150	224.5	380.2	393.6	49.2	155.7	(2.16)	3.16	(0.44)	59.4
	150	225.0	376.0	384.5	48.1	151.0		3.14		59.6 (0.24)
Casein 2/3 Intake	150	225.0	328.0	275.0	34.4	103.0	(1.49)	3.00	(7.74)	65.6
	150	221.4	326.6	251.7	31.5	105.2		3.34		71.0 (5.59)
Fish Meal	150	225.0	445.7	598.4	74.81	220.7	(4.86)	2.95	(4.40)	53.8
	150	224.0	453.3	584.9	73.11	229.3		3.14		56.6 (3.59)
Cottonseed Meal	150	224.0	295.1	279.1	34.8	45.2	(27.22)	1.30	(25.79)	36.8
	150	224.0	275.4	283.3	35.4	66.7		1.88		46.1 (15.87)
Wheat Germ	150	224.0	252.0	271.2	24.7	28.0	(15.52)	1.13	(14.68)	24.8
	150	224.5	260.4	275.1	25.1	34.9		1.39		27.6 (7.56)
Beef Liver	150	222.9	440.0	689.4	74.9	217.0	(4.87)	2.90	(4.02)	50.4
	150	225.0	458.5	699.4	76.0	233.5		3.07		53.0 (3.56)
Brewer's Yeast	150	223.5	265.0	299.9	37.5	41.5	(4.30)	1.11	(10.04)	28.0
	150	220.5	264.6	275.8	34.5	44.1		1.28		30.4 (5.82)
Defatted Whole Egg	150	224.1	305.6	332.1	42.7	81.5	---	1.91	---	48.0
	150	223.7	289.6	196.6	24.6	52.9*		---		---
Defatted Salmon Egg	150	223.3	394.1	420.6	52.6	170.8	(0.04)	3.24	(6.26)	53.7
	150	223.0	393.9	434.5	54.3	170.9		3.15		52.0 (2.27)
Non-Protein	150	222.8	199.7	241.2	---	---	---	---	---	---
	150	223.5	189.3	279.6	---	---		---		---

\*Terminated at the end of 4 weeks, due to outbreak of Kidney Disease.



Table 7:--Proximate analysis data

Diet	Experiment 1				Experiment 2			
	H <sub>2</sub> O	Prot.	Fat	Ash	H <sub>2</sub> O	Prot.	Fat	Ash
Casein-Ad Lib	78.9	60.2	26.7	9.8	77.2	60.0	31.0	9.1
" "	77.9	59.0	29.7	9.6	76.8	59.1	29.7	9.7
Casein 2/3	78.8	62.6	25.1	10.3	77.5	61.4	28.6	9.9
" "	79.3	60.8	27.0	9.9	77.6	61.9	25.4	10.1
Fish Meal	77.9	58.5	34.3	9.3	75.4	58.9	33.6	9.4
" "	78.1	59.4	32.7	9.4	75.9	57.4	33.6	9.2
C.S.H.	79.9	58.3	27.9	10.1	77.4	54.3	33.4	10.0
" "	79.2	57.7	30.8	10.3	74.1	55.0	32.9	10.0
Wheat Germ	79.8	61.9	29.8	11.0	78.0	58.9	30.3	10.6
" "	78.7	59.5	31.1	10.8	77.3	56.5	31.9	10.8
Beef Liver	75.7	54.8	35.4	9.0	73.3	51.5	39.1	8.4
" "	76.7	54.6	37.2	8.1	73.2	51.0	40.0	7.7
Brewer's Yeast	79.6	59.0	30.8	10.6	78.0	56.7	29.6	11.0
" "	79.2	58.9	31.3	10.4	77.8	57.2	29.7	10.4
Defatted Whole Egg	77.9	54.8	33.2	9.9	76.1	59.3	32.2	9.5
	77.6	54.2	34.6	9.5	76.3	59.0	30.8	9.0
Defatted Salmon Egg					77.3	56.0	32.8	9.0
					77.3	58.2	30.6	10.3
Soybean Meal	90.0	75.4	14.8	15.3				
	83.4	70.1	18.3	14.3				
Non protein	80.4	59.5	21.8	11.3	79.9	57.4	26.3	11.0
" "	80.8	60.5	21.2	11.4	78.8	56.0	24.6	11.9
" "	79.7	61.5	22.9	11.3				

An experimental diet and regimen (based essentially on rat techniques) can be utilized in the assay of dietary ingredients in fish rations. Requisites of a standard procedure listed by Chapman and Derse to measure Protein Efficiency Ratio have been met in most details. The experimental basal ration described in this report is a modification of the test diet routinely employed at this laboratory, which has successfully sustained rainbow trout for two complete life cycles and which also has been fed to a variety of other salmonid fishes. It is believed to be a reasonably complete diet since sexual maturation, egg production and viability were normal. The diet was well received by salmon when fed without a protein supplement and thus permitted calculation of NPU. The addition of a non-protein diet comparison in more refined

nitrogen balance studies is, of course, obvious. Casein proved to be of high nutritive value and this could be used as a reference standard.

A six-week assay period was followed in this trial although a preliminary adaptive period on the test diet could be employed to shorten the experimental interval. Small individual fish can not be employed but large numbers of fish permit randomization within lots. Weight gains are easily determined and records of diet consumption readily maintained. Variation in body composition is not apparent from PER data. However, simple moisture and protein analysis of individual fish can be used to calculate NPU.

Certain limitations are involved in this method, particularly with salmon, because young

fry are available for a short period only. Rainbow trout fry can be obtained at more frequent intervals due to varying spawning habits, and are generally easier to maintain. Ad libitum feeding is not feasible; only relative effects of varied intake can be measured. Precise determinations of diet ingested cannot be made. However, the effect of restricted intake does not appear to affect the fish adversely, except to reduce growth rate. Efficiency of utilization may even prove to be enhanced and wastage of feed is minimized by increased avidity of feeding. The age of the population may also be a factor since generally higher NPU values were found with older fish although PER values were quite similar.

#### Carbohydrate digestibility coefficients

Dr. William Ellis of the Department of Animal Nutrition at Texas A & M has offered to cooperate with the laboratory on preliminary measurements of the apparent digestibility coefficient of various carbohydrate sources used in experimental and practical rations for salmon and trout. The recent development of a metabolic chamber for collecting independently, respired nitrogen wastes, urinary wastes and fecal waste material has enabled us to force feed individual fish various dietary ingredients and measure the amount of any particular nutrient which is digested, absorbed and utilized. Dr. Ellis has extensive experience in measuring improved carbohydrate availability and digestibility coefficients for cattle and many other animals using relatively unavailable carbohydrate sources as carbon energy supplies. When the 5 metabolic chambers currently in use on other experiments become available in January fish will be force fed with a number of experimental diets containing various carbohydrate sources. Fecal material will be carefully collected and preserved for subsequent assay of available carbohydrate material and carbohydrate conversion by Dr. Ellis and his group at Texas A & M.

### PHYSIOLOGY

#### Salmon and trout thyroid functions

The experimental design for thyroid function studies in chinook salmon and rainbow trout

was described in the Annual Report for 1962. With the development of refined sampling techniques, adoption of micro-serological blood chemistry procedures and the addition of a new scintillation crystal more sensitive to the low energy  $I^{125}$ , preliminary studies have now been initiated or completed in the young chinook salmon, rainbow trout, and adult spring chinook salmon. Similar populations of the steelhead trout were lost in the early spring from kidney disease. Examination of 16-month-old salmon on a low iodine diet in May revealed that thyroidectomy was not complete six months after treatment of six bi-monthly intraperitoneal injections of  $100 \mu I^{131}$ . However it was noted that radioiodine  $I^{125}$  turnover of these animals had a significant 2/3 reduction (as compared to control fish) in iodine concentrating facilities.

Analysis of the thyroid region and blood fractions from December samplings of the young chinook is currently being made. Gross observations of the  $I^{131}$ -treated fish did not reveal significant developmental changes of the body characteristics that were associated with chronic hypothyroidism in the young rainbow trout. December weighings of the  $I^{131}$ -treated juvenile chinooks and their controls revealed no divergence in growth rates; average weight of the  $I^{131}$ -treated fish was 59 grams compared with 61 grams for the control diet fed group.

The last of the thyroidectomized rainbow trout and associated controls, received as eyed eggs in November 1960, were sacrificed in May, 1963. Results obtained from thyroid and blood analysis did not differ materially from those previously reported. Growth differences and malformations between the two groups were dramatically accentuated. Average weight of the thyroidectomized fish was 143 grams reflecting a 14 percent gain in weight in the last eight month period whereas the control fish had a 78 percent weight increase to 343 grams (Figure 6). Observed retardation of cephalic development between the thyroidectomized fish and their controls was quite impressive at this time but upon chemical analysis were shown not to have significant increases in phosphate or sulfate concentration in the structural tissues of the head of the thyroidectomized when compared to control fish. (Table 8). Heads were severed at the base of the skull. Eyes, brain and any

apparent connective tissue were removed. The remaining cartilagenous tissue and skin was reduced to dry weight and then was ashed in a muffle furnace at 600°C. Motion pictures were taken of animals from each group for later analysis of suspect neurological disorders resulting from thyroidectomy or from secondary brain damage due to radiation.

Iodine metabolism studies in the adult spring chinook consisted principally of two phases in 1963: (1) thyroid function during sexual maturation; (2) a pilot study in radiothyroidectomy of sexually maturing adult salmon. All phases were hindered by an unusually heavy mortality resulting from repeated handling and parasitic erosions.

#### Radiothyroidectomy of adult salmon

Radiothyroidectomy was attempted in ten migrating adult fish captured in April, by five intracardiac injections of 5 mc  $I^{131}$  each (as carrier-free iodide) over a four week period. Resulting thyroidectomy was expected by mid-summer. Mortality in the  $I^{131}$ -treated fish limited the subsequent  $I^{125}$  turnover studies and analysis to three animals in September.

Nevertheless, it was possible to estimate that a 90 percent reduction in functional thyroid tissue was achieved by this treatment. Additional studies would elucidate the thyroidal and peripheral metabolism of iodine in adult salmon as well as provide an understanding of thyroidal involvements associated with gonadal maturation, aging, and redistribution of tissue iodine reserves in the absence of the reparative effects of food intake, including iodine.

Primary emphasis was again placed upon the iodine metabolism during freshwater maturation of the adult chinook. Particular attention was given to thyroid function and iodine metabolism upon their early entry into the freshwater in early April and then again in late August-early September as the fish completed sexual maturation. Although similar uptake patterns in the thyroid gland (percent injected dose/thyroid region) were obtained as last year, iodine concentrations were higher in April (65  $\mu g I^{127}$  per thyroid region) than in May of last year (39  $\mu g I^{127}$  per thyroid region). In contrast, there was no significant difference in stable iodine content of the various blood fractions at the two times of capture. Whether this is indicative of a phenomenon dependent entirely upon

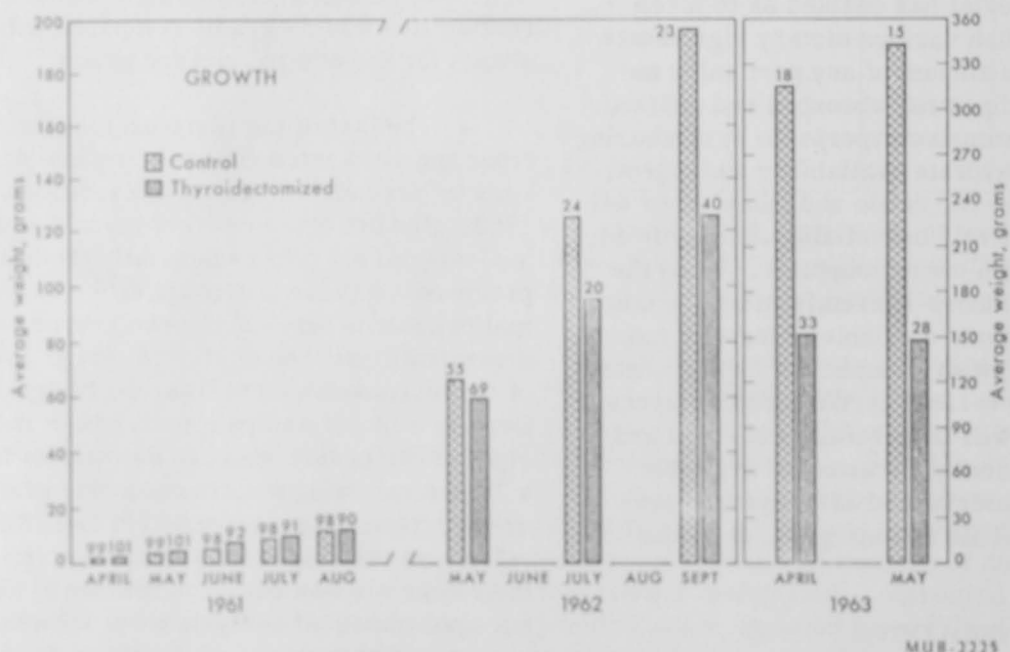


Figure 6:--Growth of control and thyroidectomized rainbow trout

Table 8:--Trout Head Analysis

	Grams	Grams	Phosphorus		Sulfur	
	Dry weight (D.W.)	Ash Weight (A.W.)	mg P/ D.W.	gram A.W.	mg S/ D.W.	gram A.W.
Thyroidectomized	1.617	0.5805	66.9	178.8	2.23	6.08
Low Iodine	3.739	0.9694	45.6	173.8	1.27	5.18
Supplemented	9.999	2.6664	48.7	173.8	1.23	4.43

Table 9:--RBC, Hemoglobin and Hematocrit values of Adult Spring Chinook in April and late August-early September 1963

Time of Sampling	Sex	Number	Red Blood Cell		Hemoglobin		Hematocrit	
			Counts	S.E.	Grams	S.E.	Percent	S.E.
April	Female	21	165x10 <sup>4</sup>	2.85	13.20	0.35	49.9	1.49
	Male	11	169x10 <sup>4</sup>	5.47	13.07	0.34	52.3	2.46
August-September	Female	20	140.8x10 <sup>4</sup>	4.34	11.12	0.25	47.3	4.36
	Male	8	151.3x10 <sup>4</sup>	8.49	11.17	0.38	48.1	2.06

the length of time in freshwater regardless of time of entry or maturation, should be determined by future studies.

Table 9 shows a breakdown of blood values for the spring and fall samplings. The only significant difference was in the RBC of females between April and August-September.

Table 10 shows blood iodine concentrations during the first month following capture at the Bonneville Dam in April. The most significant decrease in blood iodine content occurred during the four weeks following the fish's initial capture in freshwater. Data on iodine metabolism in the fall samples will be compiled, analyzed and presented in a future report.

#### Chemical water differences and stream survival of trout

A description of this project and preliminary findings were presented in the 1962 report.

Analysis including serum electrolytes, tissue electrolytes, intact carcass analysis for protein, fat, ash, moisture, and major inorganic ions are now complete with the exception of the samples taken at the termination of the field experiment. Evaluation of this voluminous data will be undertaken on completion of the final analysis. Preliminary trends suggest a failure of the fish to maintain serum electrolyte concentrations with increasing debility; a marked increase in both tissue and total body moisture and a shift in inorganic distribution with debility.

#### Copper and zinc versus embryonic development of salmon

During the current egg hatching period experiments are being conducted to (a) confirm the previous years findings (b) to establish the lower level of copper toxicity (c) to determine the period of maximum sensitivity to copper and zinc toxicity. Incubation and maintenance

of metal ion concentrations was accomplished essentially as in previous years. The incubation design is illustrated schematically in figure 7. Referring to the figure, bottles 1-6 receive no copper or zinc, bottles 7, 8, and 9 receive 2.5, 5, and 10 ppb Cu respectively, bottle 10, 50 ppb Zn; bottles 11, 12, and 13 receive 2.5, 5 and 10 ppb Cu in addition to 50 ppb Zn, bottles 14-18 also receive 10 ppb Cu and 50 ppb Zn while bottle 18 received 20 ppm Calcium also. As the previous year's study indicated the period of maximum sensitivity was after the eyed egg stage, the current year's work was started with eyed eggs. Varying periods of exposure to copper and zinc ions was accomplished by exchanging bottles 3 and 14, 4 and 15, 5 and 16 and 6 and 17 at approximately 100 temp unit intervals. Metering rates and flow rates were checked frequently during the experiment to obtain an estimate of the actual concentrations during the treatment period. Samples of eggs were taken of approximately 100 temp unit interval to determine the uptake and retention of copper and zinc. An evaluation of kind and intensity of white spot and other abnormalities was made by examining a representative sample of each group at 100 temp unit intervals after hatching. Five more or less distinct types of white spot were defined which are:

- (1) a "cottony" appearing ring around the small oil globules on the surface of the vitelline membrane;
- (2) small white flecks either attached to the membrane or inside the yolk material, not associated with oil globules;
- (3) white spots within the main oil globule (very seldom seen);
- (4) strands of "cottony" appearing material on the inner surface of the vitelline membrane or inside the yolk (this may be a development of type 2 as both types are frequently seen together);
- (5) a large chunk of white material within the yolk. Types 4 and 5 are most frequently seen in affected hatchery fry,

we feel that these, rather than 1, 2, and 3 result in death to the fry.

The types are illustrated in figures 8 and 9.

Analyses for retained copper and zinc have not been completed. Data regarding incidence, type and severity of white spot has not been tabulated and evaluated. Table 11 summarizes the results of hatching and mortality rates during the experiment until December 30. The acceleration of hatch with increasing concentrations of copper was significant and even more pronounced than the summary, which presents only temperature units to mean hatch, indicates. Significant mortalities occur even at 2.5 ppb copper. This was surprising in that it is roughly 10 times more dilute than the level previously found to result in acute toxicity to chinook fingerlings.

An evaluation of the synergistic effect of zinc and copper (which was clearly indicated last year) must await final determination of concentration ranges within the experimental period. The observation that the yolk fry are far more sensitive to copper ion than are the eggs--note groups 3-6 vs. groups 14-17--was unexpected in view of the pronounced effect which copper ion has on hatching rate and the appearance of the hatched fry. In contrast to the accelerated hatch the copper ion markedly retards the rate of fry development. Treatment was discontinued and all fry were transferred to troughs at 139<sub>3</sub> temperature units when the controls and group 10 and 14-17 were starting to "swim-up". The fry under copper (or copper and zinc) treatment, however, showed few signs of side-wall development. This was illustrated in last year's report. As before the fry will be held under observation for sufficient time to determine any continuing effects of the treatment.

A new group of yolk fry have been obtained and placed under treatments as above. This experiment is being conducted to determine the toxicity of the various levels to previously untreated fry and to identify the time of maximum sensitivity more closely.



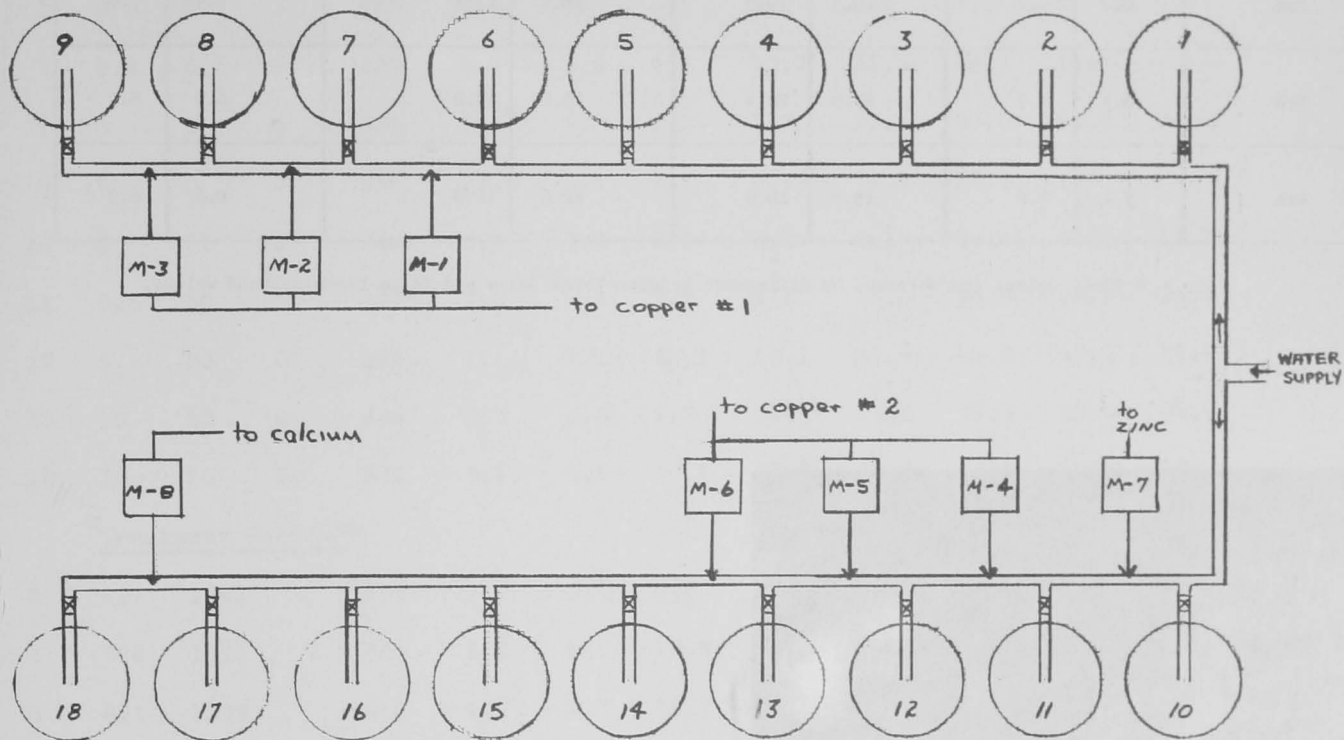


Figure 7:--Schematic representation of incubation design for determining the effects of copper and zinc on embryonic development. Numbered circles represent bottle incubators, squares represent pumps which meter the concentrates into the water line at the points indicated. See text for treatment description

Table 10:--Spring chinook blood iodine during first month following capture at Bonneville Dam  
 $\mu\text{gm I}/100\text{ ml}$

Week After Capture	Whole Blood		Total Serum		Serum Protein-Bound		Serum Iodide*	
	♂	♀	♂	♀	♂	♀	♂	♀
1st	16.1	15.6	43.5	40.0	25.8	21.9	17.5	18.1
2nd	8.2	8.6	25.5	19.5	18.4	11.7	4.9	7.8
3rd	6.3	6.7	21.9	20.4	15.0	14.2	6.9	6.2
4th	5.1	7.2	15.2	19.0	12.6	10.8	2.6	6.2

\* These values are obtained by difference between Total Serum and Serum Protein-Bound values.

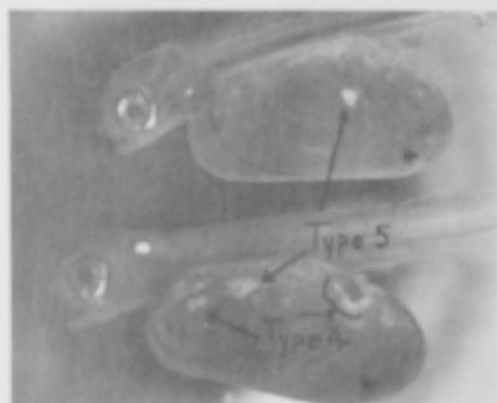


Figure 8:--Chinook salmon fry showing various types of white spot ( 2 photos)

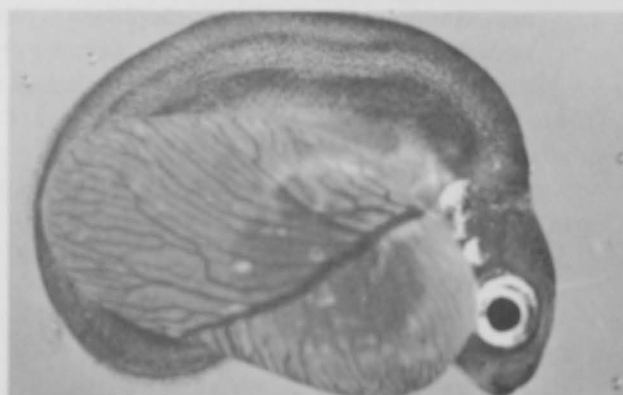


Figure 9:--Abnormalities increase in frequency with copper and zinc exposure during egg development.

Table 11:--Effect of copper and zinc on mean hatch and mortality rate of chinook salmon

Lot No.	Treatment			T.U. mean hatch	egg*	Mortality*						
	Cu.	Zn	Ca			fry						
	ppb	ppb	ppm			970	1054	1117	1203	1290	1393	1495
1	0	0	0	887	9.2	4.1	4.1	4.7	6.8	9.0	10.2	12.9
2	0	0	0	887	4.9	3.0	3.1	3.1	3.2	3.2	3.7	4.4
7	2.5	0	0	883	6.2	7.9	9.4	12.7	31.4	53.5	lost	-----
8	5	0	0	881	11.1	5.5	6.1	7.3	22.1	57.0	68.2	94
9	10	0	0	856	7.0	8.1	9.7	22.7	76.0	87.1	100	
10	0	50	0	884	5.4	3.7	6.2	8.2	9.1	9.1	9.7	11.7
11	2.5	50	0	887	7.0	3.4	7.2	9.1	15.0	20.8	24.2	25.5
12	5	50	0	878	12.1	10.2	15.1	19.1	32.9	44.9	68.2	79.4
13	10	50	0	864	8.3	3.6	4.7	7.0	23.2	39.3	59.4	85.4
18	10	50	20	878	5.7	9.1	10.6	15.7	64.3	96.5	100	100
<u>Treatment Period**</u>												
3	627	1393		864	7.1	5.1	6.6	8.2	18.2	39.2	59.3	82.9
4	728	1393		883	5.2	6.2	10.4	16.3	38.8	62.6	83.3	94.0
5	821	1393		888	6.7	8.7	14.5	32.8	58.6	75.7	87.5	93.5
6	953	1393		888	5.3	3.1	3.8	6.9	53.5	82.4	86.7	93.9
14	522	627		885	4.6	5.1	6.8	11.4	14.7	16.3	18.7	20.3
15	522	728		881	6.3	4.1	4.5	4.9	5.4	6.0	6.3	8.3
16	522	821		855	4.8	4.4	4.9	5.0	5.1	5.1	6.0	6.4
17	522	953		859	4.5	4.3	6.6	7.0	7.1	7.5	7.9	8.3

\* Egg mortality based on percent of eggs started, corrected for samples removed. Fry mortalities represent accumulated mortality at the temperature units indicated the percent is based on the number of hatched fry corrected for fry sampled through 1400 temp. units.

\*\* Eggs or fry were incubated with water containing 10 ppb Cu and 50 ppb Zn during the period indicated by the temperature units, during the balance of the time they were in untreated water.

## Toxicity of zinc to salmonid fishes

Concentrations of zinc which will produce acute toxicity were determined for rainbow trout and chinook, silver and blueback salmon fingerlings. Acutely toxic concentrations of zinc were arbitrarily defined as that concentration which would result in death to half of the starting population within 5 days. Tests were conducted in hatchery troughs supplied with well water at a rate of 10 liters per minute. The water is soft (TDS=35 ppm), slightly acid (ph=6.8) and was held at a temperature of 10°C. Water flow to each trough was maintained with self-regulating, constant flow valves while zinc ion concentration was maintained by metering a reagent grade zinc sulfate concentrate into the pipeline supplying each trough at a constant rate. Tests were conducted with 3 to 4 levels of zinc and negative control simultaneously, each lot under test consisted of 100 fish, of 3 to 5 gm average weight. All tests were repeated at least once, confirmatory runs were generally in good agreement. Results are summarized in table 12.

Table 12:--LD<sub>50</sub> time versus dose for zinc

Species	Zn Conc. ppm					
	0.1	0.2	0.3	0.4	0.5	2.0
Chinook	*	40 hrs	32			
Silver	*	96	66		48	40
Blueback		76	70	48	40	
Rainbow	*	72	64	30	21	

\* Less than 24% mortality

An experiment was conducted to produce and measure the effects of chronic zinc toxicity in chinook salmon. Four groups of 1,000 chinook salmon fingerlings were raised in water containing 0, 0.033, 0.067, and 0.10 ppm zinc for a 16-week period. Water conditions, flow and temperature were as described in the acute toxicity studies. Zinc concentrations were held constant by metering as before, the fish were fed a complete test diet and were maintained in accordance with standard feeding trial procedures of this laboratory. At the end of the 16-week period samples were taken to determine zinc accumulation, standard hematological parameters

were measured and respiration rate was determined on replicate samples from each group. Approximately half of the population in each group was transported to the Salmon Cultural Laboratory for stamina tests by Burrows and his staff and the remaining fish were taken off treatment and held for an additional 8 weeks. Triplicate samples were taken from each group at weekly intervals to determine loss of accumulated zinc.

No significant difference between groups was determined for weight gain, percent mortality, respiration rate or stamina performance. Results of zinc retention are presented in table 13. The direct relationship between zinc accumulated and zinc concentration to which the fish were exposed is striking. The strong retention for such an extended period of time suggests that much of the zinc is probably laid down in the bone matrix rather than the soft tissues where excessive quantities would be easily exchanged.

Table 13:--Zinc in chinook salmon after 16 weeks exposure.

Exposure level	0	0.033 ppm	0.067 ppm	0.10 ppm
Weeks after termination				
0	76*	150	195	253
3	90	130	165	217
5	87	130	137	206
8	89	109	132	212

\*Concentrations in ug per gram moisture free fish.

## Hepatoma I induction study

Microscopic examination of intermediate and terminal samples of livers from rainbow trout fed different levels of 13 chemical carcinogens and fat, protein and carbohydrate fractions of the suspect commercial feed have been completed. Basophilic staining tumors were found in fish fed each of the 13 chemical carcinogens and in those fed the lipid, protein and carbohydrate subfractions of the feed. Adenocarcinoma, trabecular carcinoma, mixed adeno- and trabecular carcinoma, extending to the advance anaplastic parenchymal cell neoplasm stage were observed. Occasionally cholangioma was observed in some samples. The incidence varied

among groups of fish fed the different chemicals at different levels and the chemical insult sometimes reflected the type of liver changes observed. Typical hepatoma was very high in the lipid fraction fed fish, low in the protein fraction fed fish, and extremely low in the "carbohydrate" fraction fed fish, during and up to the final 20 month terminal examination. The list of potential rainbow trout carcinogens summarized in the 1962 Annual Report can therefore be extended to include thioacetamide, aminotriazole, and diethylstilbesterol, and the reports on the other compounds were reinforced from these additional analysis of the terminal samples.

### Hepatoma II induction study

The second hepatoma induction study was completed and terminal samples were collected after 20 months of feeding 9 chemical carcinogens at that level previously found positive for the appearance of hepatoma, and at a correspondingly lower level. In addition, feeding studies with the lipid, protein and carbohydrate fractions were repeated. New studies challenging fish with neutral lipid and phospho lipid components from the fat from commercial suspect ration were included in the design of the experiment and results were compared with those fed the original pellet (supplemented with vitamins) and the complete test diet negative controls. Initial feeding rainbow trout fry were fed the complete test diet with and without carbarsone, thioacetamide, aminotriazole, 2-acetyl-amino-fluorene, aminoazotoluene, DDT, para-dimethyl-aminoazobenzene, dimethylnitrosamine and diethylstilbesterol at two levels to duplicate lots of 100 fish each contained in small troughs supplied with constant temperature (15°C) water. Fish were fed three times daily, 6 days weekly at about 90 percent of ad libitum acceptance level until three grams in size at which time they were fed at the same rate but twice daily, 6 days weekly, until 6 months in age. At that time all duplicate groups were combined and the population reduced to 100 fish for each treatment. A large-scale selection of samples for subsequent histological analysis was obtained from the extra fish in each treatment. Internal organs of all fish sacrificed at this time were closely examined and anomalies or any suspect

differences were recorded to correlate with later histological findings.

Fish (100) from each of the combined lots were then continued on each treatment in six-foot circular tanks. The feeding frequency was gradually reduced to once a day 6 days per week for the larger fish. After 12 months of feeding each fish was surgically inspected and the specific characteristics of livers and other internal organs was described and recorded on a card of each number-tagged fish. Representative samples were selected for each type of liver lesion and anomaly observed, and the remainder of the population was continued on the treatment. After 16 months of feeding each fish was again surgically inspected and any changes or new anomalies in each liver or any other internal organs were recorded. Again representative samples of each condition were prepared for subsequent histological analysis. Non-selected survivors were continued on the treatment for an additional four months. After 20 months of feeding the various carcinogens or fraction containing diet, each fish was again surgically inspected, liver and other internal organ changes were recorded, and 10 fish with liver tumors, 10 fish without liver tumors, and 10 representative random fish from each treatment were preserved for subsequent histological analysis. All other livers were frozen for future chemical analysis and certain advance metastasizing specimens were preserved for specific biochemical or histological tests. In these studies grossly visible tumors were observed in fish challenged with each level of each chemical carcinogen, with each fraction and each subfraction of the suspect commercial ration, and in those fish fed the original pellet with the vitamin supplement. The only lots negative were those groups fed the complete test diet at the 6, 9, 12, and 16 month inspection periods.

The first fish examined in the CTD control lot at the 20 month feeding period contained a small classical appearing liver tumor. All other fish examined in this lot (70) were negative for typical liver tumors. Large numbers of typical hepatoma-like liver tumors were observed in those groups of fish fed the total lipid fraction, the neutral lipid fraction and the suspect com-



mercial pellet with the vitamin supplement. Small numbers of hepatoma-like tumors were seen in those groups fed the protein, carbohydrate, or phospho lipid fractions from this feed. All fish fed the high level of thioacetamide were blind by the end of the experiment. Diethylstilbestrol-fed fish all died within 5 months after the start of the experiments at the lowest level fed. Other specific effects of toxic or chronic anti-metabolic physiological activity were observed in fish fed other compounds. These terminal samples will be analyzed in 1964.

### Hepatoma induction III

Aflatoxin fractions, neutral lipid fractions alpha, beta, gamma, delta, and epsilon and polymerized non-urea adduct forming fat with and without dimethylnitrosamine and 2-AAF were fed to groups of young rainbow trout. In addition, confirmatory feeding trials were designed for fish fed 6 percent of total lipids, 5 percent of neutral lipids, the pellets plus vitamin supplement controls, 2-AAF, DMN and stripped and oxidized herring oils. Details of the groups on experiments can be seen in table 14. The aflatoxin fractions were prepared from *Aspergillus flavus* Link X Fries cultures grown on cracked wheat and the mycotoxin separated into pure aflatoxins B and G with thin layer chromatography by Dr. Wogan at MIT. Aflatoxin B and G were compared with those obtained from *A. flavus* contaminated peanuts and with crude concentrate obtained from stripping wheat and peanuts with chloroform, expelling the solution into an excess of petroleum ether, and then removing and concentrating the flocculent precipitate of aflatoxin-containing material.

Samples of pure aflatoxin B and G received from MIT were analyzed for fluorescent activity at the Sixth Army Area Medical Laboratory. The pure compound activated at 375 $\mu$  and fluoresced at 430 $\mu$ . Activation scanning of chloroform suspect diet ingredients which were common denominators in feeds for fish with lateral line hemorrhagic syndrome activated at 375 $\mu$  but fluoresced at 450 $\mu$ . Subsequent ducklings assays conducted at MIT showed some liver changes, but the typical aflatoxin syndrome was not observed.

Young trout fed 5 percent of the dry diet of non-urea adduct forming polymerized fat died within three weeks. Fish fed one percent of the material continued to grow, but at a reduced rate for an additional four months. Only a few survivors were still alive at the six month sampling period. After six months of feeding the hepatoma induction diets to paired groups of 100 fish each, all fish on each treatment were combined, the population reduced to 150 per group, and each group was transferred into 6 foot circular tanks for further study.

Diet preparation, feeding and other experimental techniques in the course of this set of experiments were similar to those used in hepatoma 1 and 2 induction tests. Internal organs of all extra fish from each treatment (excess of 150) were examined closely for anomalies. Any liver or other internal organs bearing a small tumor was individually characterized and preserved for subsequent histological analysis. In addition ten individual random samples of livers from each lot examined was preserved for later close microscopic examination. No survivors were present in any of the lots fed the non-urea adduct forming fat and therefore the 2-AAF positive control for this group was terminated at six-month examination. Small tumors were observed in a number of livers in fish fed crude aflatoxin concentrate.

Subsequent histological analysis confirmed the presence of a high incidence of trabecular hepatoma in these samples. Only one grossly visible tumor was observed in the total lipid fraction fed group of fish although subsequent microscopic review revealed classical hepatoma in this fraction, in the neutral lipid fraction, and in the beta subfraction of the neutral lipid fraction. All other groups of fish given the different treatments have been continued and are scheduled for further examination for hepatoma after nine months of feeding (January 1964). Microscopic analysis of the internal organs from the samples already obtained will continue. Final analysis of these samples will entail a number of months for review.

Table 14:--Hepatoma Induction III

<u>Lot Number</u>	<u>Diet Number</u>	<u>Carcinogen</u>
128 & 129	117	6% Fat Fraction
130 & 131	130	Neutral Lipid
132 & 133	132	Pellet Control
134 & 135	118	CTD Control
136	154	Aflatoxon B-1 1/4X (.0005 ppm)
137	155	Aflatoxin B-1 1X (.002ppm)
138	156	Aflatoxin B-1 4X (.008 ppm)
139	157	Aflatoxin G-1 1/4X (.0005 ppm)
140	158	Aflatoxin G-1 1X (.002 ppm)
141	159	Aflatoxin G-1 4X (.008 ppm)
142	160	Aflatoxin-Peanut Fraction b-1 1/4X (.005 ppm)
143	161	Aflatoxin-Peanut Fraction b-1 1X (.02 ppm)
144	162	Aflatoxin - Peanut Fraction b-1 4X (.08 ppm)
145	163	Aflatoxin - Peanut Fraction g-1 1/4X (.005 ppm)
146	164	Aflatoxin - Peanut Fraction g-1 1X (.02 ppm)
147	165	Aflatoxin - Peanut Fraction g-1 4X (.08 ppm)
148 & 149	166	Aflatoxin - Crude Extract 1/16X (.00125 ppm)
150 & 151	167	Aflatoxin - Crude Extract 1/4X (.005 ppm)
152 & 153	168	Aflatoxin - Crude Extract 1X (.02 ppm)
154 & 155	169	Aflatoxin - Crude Extract 4X (.08 ppm)
156 & 157	170	Aflatoxin - Crude Extract 16X (.32 ppm)
158 & 159	172	Polymerized Fat 1% plus DMN 1/4X plus 4% C.O.
160 & 161	173	Polymerized Fat 5% plus DMN 1/4X
162 & 163	174	Polymerized Fat 1% plus AAF 1/4X plus 4% C.O.
164 & 165	175	Polymerized Fat 5% plus AAF 1/4X
166 & 167	176	Polymerized Fat 5%
168 & 169	177	AAF 1/4X plus 5% Corn Oil
170 & 171	178	DMN 1/4X plus 5% Corn Oil
172 & 173	133	Oxidized Herring Oil 6%
174 & 175	135	Stripped Herring Oil 6%
176 & 177	179	Neutral Lipid Fraction - Alpha 2.6%
178 & 179	180	Neutral Lipid Fraction - Beta 0.9%
180 & 181	181	Neutral Lipid Fraction - Gamma 0.6%
182 & 183	182	Neutral Lipid Fraction - Delta 0.8%
184 & 185	183	Neutral Lipid Fraction - Epsilon 0.2%
186 & 187	118	C.T.D. Control

#### Hepatoma Induction IV study

Intense activity has been directed on further fractionation of the alpha subfraction from the neutral lipid fraction from the fat from the suspect ration, on the basis that the alpha component was the major constituent in the neutral lipid fraction. This material was subfractionated into five sub units and functional group analysis was completed on these residues. With the tentative observation that the beta sub-fraction fed groups of fish did contain some individuals with hepatomatous livers after six months of feeding, major research efforts were shifted to the beta subfraction. Six silicic acid column

chromatography separations using various concentrations of normal hexane and diethyl ether ratios as the eluant have been completed. The beta subfraction has been separated into four or five major components. Further work is necessary for more discrete separation with specific solvent ratios to assure reproducible subcomponents for inclusion in the bioassay in the hepatoma IV experiments. In addition to the beta subfractions, the alpha, beta, gamma, delta, epsilon fractions of the neutral lipid components; confirmatory feeding trials for more aflatoxin components; and more non-urea adduct forming fat tests have been included in the design for the new feeding studies planned to start late in January 1964.

Dr. Elizabeth Weisburger of the National Cancer Institute has synthesized and furnished us with N-hydroxy acetyl aminofluorene for a comparison study with 2-AAF in the diet of trout. New samples of duckling assayed crystalline aflatoxin B<sub>1</sub> and aflatoxin G<sub>1</sub> have been received from MIT. Samples of non-urea adduct forming polymerized fat have been obtained and will be compared with a commercial compound of similar characteristics. In a very large scale study with the National Cancer Institute, USDA, National Cotton Producer's Association and the Ralston Purina Company, a number of cottonseed meals prepared by three standard processes have been selected from various parts of the country and have been incorporated into a commercial type pellet for feeding young rainbow trout at a commercial hatchery. Samples from five of these cottonseed meals were processed at the USDA regional laboratory in New Orleans; Dr. Leo Goldblatt removed the fat from these five meals using the Bloor-LaRoche solvents in a special-design continuous extractor. When we received them from New Orleans, the fat concentrates were washed, the "carbohydrate" components removed and final solvent residues evaporated in vacuum. After assay of the total fat present in these meals and in the samples incorporated in the commercial products have been completed these respective levels of the fats contained in these cottonseed meals will be incorporated into the complete test diet, for the studies we plan to begin in January.

Final plans for hepatoma induction IV studies have not been completed, but the current tentative design can be summarized as follows: Aflatoxin B<sub>1</sub>, G<sub>1</sub> and crude material at three different levels; alpha, beta, gamma, delta, epsilon groups; beta 1, beta 2, beta 3, beta 4, beta 5 subgroups; phospho lipid at 1 percent of ration; five cottonseed meal fats; commercial polymerized fat at three levels; commercial polymerized fat plus AAF and DMN respectively; purified non-urea adduct forming polymerized fat at two levels, thioacetamide; N-OH AAF versus 2-AAF; and complete test diet controls. Space does not permit consideration of additional treatments. In addition to the above long-term feeding plan 10 female trout have been obtained in the terminal stages of ovarian development.

These fish will be challenged with high levels of aminoazotoluene and the effect of this known carcinogen upon the incidence of anomalies and upon the sensitivity of the ensuing progeny to small insults of AAF and DMN will be investigated. Under consideration in the design for future plans are tests of time-versus-dose responses to confirm and extend the report that 30-day challenges of certain carcinogens may result in a measurable incidence of neoplasms after the carcinogen has been removed from the diet.

#### Acetylaminofluorene and N-hydroxy Acetylaminofluorene intermediates

Assays by Dr. Miller at the McArdle Memorial Research Institute disclosed strange and different compounds appearing in the urine of 2-AAF and N-hydroxy acetylaminofluorene challenged rainbow trout. Less than 10 percent of the dose of either 2-AAF or N-OH AAF was recovered in urine when fish were force fed these compounds, were held in the metabolic chamber, were cannulated and the urine collected for three days after dose administration. Careful preservation of the urine with toluene and keeping it in a frozen condition until assayed did not disclose excessive oxidation of intermediate compounds. Adaptation of the existing metabolic chamber to accommodate a latex rubber dam through which fish may be inserted was designed and tested. Preliminary results indicated almost complete separation of metabolic wastes excreted through the gills, feces and ureter. Several fish were dosed with AAF or N-OH AAF by stomach intubation and the feces and urine were collected separately. Other fish were implanted with AAF or N-OH AAF in the peritoneal cavity. Subsequent analysis of fecal and urine samples disclosed that force fed, injected, or implanted fish, most of the material appeared in the feces except for the implant samples. In force fed fish reasonable amounts of the reduced material was found in the feces. Microamounts of 5-hydroxy AAF and 7-hydroxy AAF were found in the urine of all fish challenged with these carcinogens. One fish urine sample also disclosed some 3-hydroxy AAF. The presence of these hydroxy derivatives indicates presence of normal hydroxylating detoxifying enzyme systems, but the general absence of the common mammalian

N-hydroxylase for 2-acetylaminofluorene was indicated. Only one fish did clearly excrete some N-hydroxy AAF in the urine after the insertion of 2-AAF through the body wall. Most of the fish excreted 5-hydroxy AAF and 7-hydroxy AAF. Administration of N-OH AAF led to the same metabolites and much N-OH AAF appeared in the feces from those challenged by intubation. Further analysis of more samples from fish challenged with AAF, N-OH AAF, and 7-fluoro AAF by insertion of the material through the body wall into the peritoneal cavity has been scheduled for January. In addition, whole livers preserved in dry ice will be assayed for new and different intermediates in the detoxifying systems present in these fish.

#### Aflatoxicosis in trout

High levels of crude and purified aflatoxin containing materials were received from MIT and administered to 25 gm trout at 0.8, 8.0, and 80 micrograms of material per fish per day for a five-day test period. Inconclusive results were obtained from fish treated with 0.8 and 8.0 micrograms per day. After fourteen days of observation half the fish given 80 micrograms of material per day for five days (400 micrograms total dose) died. In 3 of five fish examined large hemorrhagic areas were observed in the viscera. Subsequent histological analysis disclosed congestion in the liver and hemorrhagic areas in the internal organs, reminiscent of an anti-coagulant toxicant. A tentative LD<sub>50</sub> dose of approximately 3 mg/kg/day (15 mg/kg total dose) for the crude material for 25 gm trout under these experimental conditions was calculated. The pure crystalline material was approximately 10 times more effective.

Administration of different increments of crude or purified aflatoxin contained in a number of solvent systems to 5-day-old Japanese quail chicks was generally unsuccessful. Post mortem observations of liver and other internal organs from moribund samples could not be directly related to dose or size effect. The quail showed great sensitivity to chlorinated hydrocarbons and to other organic solvent residues but routine duckling assay techniques could not be used under our experimental conditions for confirmatory aflatoxin studies.

Activation and fluorescent wave lengths were determined for the pure crystalline compounds on a Spinco Bowman spectrofluorometer at the Sixth Army Area Medical Laboratory. Infrared absorption spectra of pure compounds was also recorded there on a Beckman IR 4, to accumulate reliable physical characteristics for these potent carcinogenic mycotoxins.

### HISTOPATHOLOGY

#### Hepatoma from aflatoxin

Samples were taken in October after 6 months of feeding five levels of crude aflatoxin (1/16X to 16X) in hepatoma induction III experiments. In approximately 130 trout sampled, eight livers were grossly suspect. On microscopic examination seven of these contained hepatoma with from one to four definite class IV nodules in each (fig. 10). Two of these were fed aflatoxin at 1/16X and five were fed at 4X levels. Subsequent examination of the remaining samples fed aflatoxin at 4X and at 16X revealed that of 30 trout fed at the 4X level 18 had hepatoma and three others had suspect nodules. Twenty six trout fed at the 16X level and examined microscopically with the skip-serial section technique only showed one nodule but other internal organ changes of congestion and diffuse hemorrhagic areas.

#### Lipid fractions

Alpha, beta, gamma, delta and epsilon fractions of the neutral lipid were fed trout for six months and histological examination at the end of this time resulted in:

Alpha - 0/27, one liver was suspect;  
Beta - 2/31, two other livers were suspect;  
Gamma - 0/41, two livers were suspect;  
Delta - 0/34, two livers were suspect; and  
Epsilon - 0/37, one liver was suspect. Classical trabecular hepatoma was observed in both positive Beta samples.

#### Polymerized fat

Twenty livers were examined from fish fed 1 percent of these components for six months. One liver showed microscopic changes suggestive of early hepatoma. No discrete nodular

formation was evident but the majority of the liver cells showed extreme vacuolation, figure 11. Scattered patches of basophilic liver cells some of which showed spindling suggestive of fibrosis were seen, figure 12. One rather extensive but somewhat diffuse area of basophilic liver cells best described as diffuse hyperplasia was evident, figures 13 and 14. It is possible that polymerized fat fed at lower levels (1/2X and 1/16X might induce hepatoma in rainbow trout since they would be less toxic.

#### Hepatoma II terminal samples

Terminal liver samples collected in October after twenty months of feeding in hepatoma induction II experiments are being processed for histological study. Many of these contained gross hepatoma nodules but since many thousands of skip-serial sections must be processed and read it will be several months before a final report can be prepared.

#### Histopathology of body wall tumors in brook trout

Samples of two body wall tumors from a brook trout reared in the National Aquarium were received in May from Dr. Hueper, N.C.I. Both tumors grew on a male fish (approx. 14 in. long) between four and five years of age. One tumor located about 10 cm. anterior from the caudal fin on the left side, measured about 5 cm. in diameter and was soft and protruding. On cut surface it was transparent and filled with mucus and fluid material. It extended deep to its point of contact with the spine. The second tumor, about 3 cm. in diameter was located about 3 cm. posterior of the right pectoral fin. Its consistency was similar to that of the first tumor, but it did not penetrate through the body wall musculature. However, it was hemorrhagic on cut surface and pink in color. Gills, liver, heart and spleen were normal.

Microscopic examination of H & E and of Masson's stained sections revealed highly degenerated body wall cavernous spaces containing mucus, blood cells, serum and alternating with areas containing strands of degenerating voluntary muscle fibers (figure 15). Additional pat-

terns of degenerating muscle fibers are shown in figures 16 and 17. The stomach contained numerous microscopically medium to large granulomatous lesions containing dark Schauman-like bodies. The lesions were extensively scattered through the submucosa and occasionally invaded muscularis and adventitial layers, figure 18. Publication of a case study is planned and the rough draft is in preparation.

#### Histopathology of pancreas tumor mass

A small spheroid firm tumor mass found attached to the pancreas and its fascia was discovered in a 14-1/4 inch male rainbow trout which had been reared in this laboratory. The tumor measured 0.6 cm. in diameter and had a well developed capsular portion partially surrounding the tumor proper which appeared to have arisen from pancreatic capsular connective tissues with included vascular tissues by having invaginated or grown into its partial capsule. Thus, the cleft between capsule and tumor proper was crescentic in cross section. Figure 9 shows the characteristic structure of a small portion of the tumor proper below, one end of the crescentic cleft above it, the wide capsule above the cleft and fatty replacement of pancreatic acinar tissue together with a blood-filled vein (above) to the left of which are two ovoid pancreatic islets (insulin forming tissue) with small groups of pancreatic acinar cells (darker colored) at the periphery of the islets. Much fibrous tissue occupied the upper portion of the central tumor mass (lower right) while its lower portion (lower left) was hemorrhagic and inflammatory in nature. Reference to Arthur P. Stout's Tumors of the Soft Tissues, Armed Forces Institute of Pathology (Section II, Fascicle 5 of Atlas of Tumor Pathology) 1953, would place this neoplasm among the hemangiopericytomas but portions of the lesion may resemble more closely a capillary hemangioma.

#### Bunny lake brook trout thirteen years of age were studied histopathologically

In all, twenty-four brook trout have been investigated by studying stained sections of most organs and tissues. Nine of these were controls collected from two California stations and one



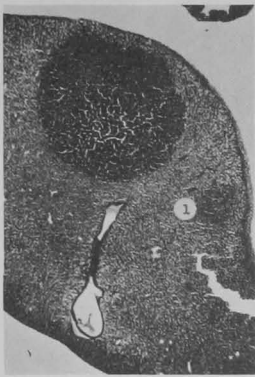


Figure 10:--Large classical hepatoma nodule (above) and small invasive early nodule (at right) of No. 1 on photomicrograph. Liver from rainbow trout fed crude aflatoxin for six months at the 4X level. X 25

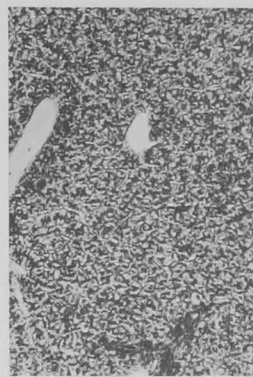


Figure 11:--Extreme liver cell vacuolation in rainbow trout fed polymerized fat 5 percent (reduced to 1 percent) plus DMN  $\frac{1}{4}$ X for six months. X 120

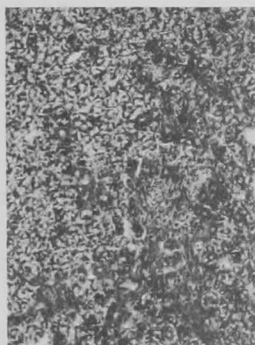


Figure 12:--Same liver as figure 2, showing patches of spindling and basophilia of pleiomorphic liver cells. X 120

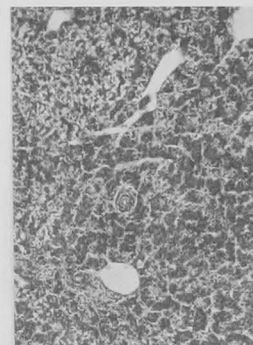


Figure 13:--Same liver as figure 2 showing part of a large area of basophilic liver cells suggesting diffuse hyperplasia. X 120

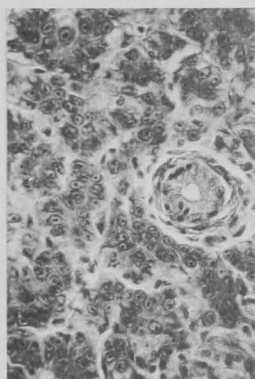


Figure 14:--Higher magnification of a portion of the field shown in figure 13. Liver cells are more or less pleiomorphic and some of them included normal mitotic figures. X 460

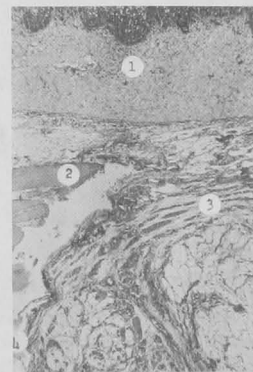


Figure 15:--Benign myxoma of brook trout body wall showing at (1) thick skin, at (2) portion of skeletal muscle fiber about normal size, (3) area around which degenerate muscle fibers may be seen, and (4) edge of area containing mucus deposits and fluid. X 50

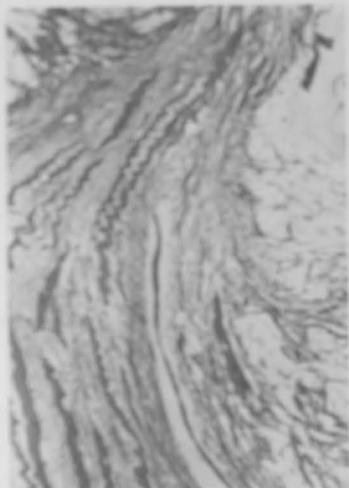


Figure 16:--Another area in the same myxoma showing degenerating muscle fibers with wavy outlines, increased fibrous tissue and mucus-filled spaces. X 50

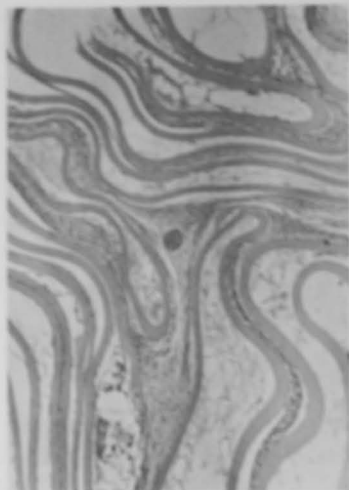


Figure 17:--Still another view of the myxoma showing abnormal pattern of degenerating muscle fibers, fibrous tissue with inflammatory cells and fluid-filled spaces. X 50

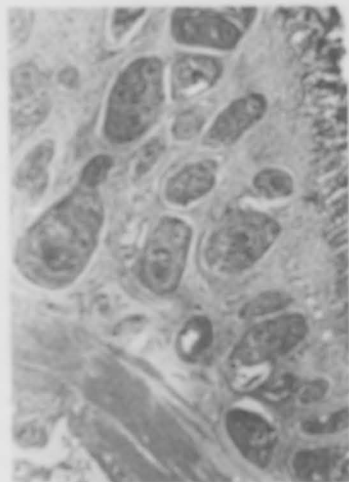


Figure 18:--Portion of stomach wall of myxomatous brook trout showing rather large granulomatous lesions scattered heavily through the submucosa. One small granuloma lies in the adventitia (outer layer) at lower left. Gastric mucosa appears along most of right side. Granulomata of the gastric wall are known to be common in brook trout from the Beaver Creek, Maryland area. However, they do not seem to cause serious harm to the fish.

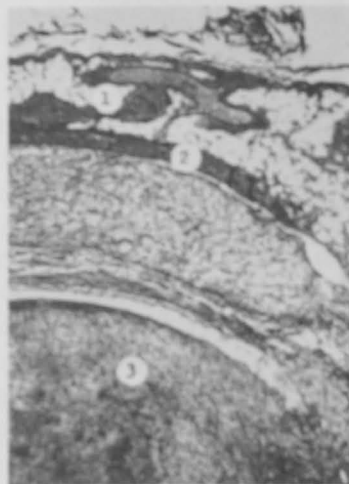


Figure 19:--Part of an hemangiopericytoma-like tumor found attached to the pancreas of a fourteen and one half inch rainbow trout (1) two ovoid pancreatic islets with narrow margins of pancreatic acinar cells and a blood-filled vein above the one on the right. At (2) is a band of dense fibrous tissue separating fatty degenerated pancreatic tissue (above) from heavily vacuolated inflammatory tissue below, the latter being continuous on the right with the main part of the tumor (3) around which are darkened hemorrhagic areas and inflammatory cells together with fibrous tissue, especially at the lower right.

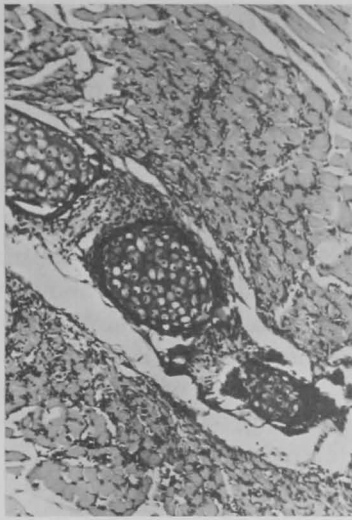


Figure 20:--Degenerating and congested pectoral fin muscle from fingerling salmon with chinook lateral line syndrome (CHILLS). Most of the muscle fibers are cut in transverse section. Sections of three fin cartillages appear between the two main muscle masses. Blood cells (black dots) appear to lie (magnification 1000X) in dilated capillaries or sinusoids, hence the use of the term congestion rather than hemorrhage. X 120



Figure 21:--Congested hypobranchial muscles adjacent to lateral line in a case of chinook lateral line syndrome. X 120



Figure 22:--Heavily congested skeletal muscles adjacent to lateral line in a case of chinook lateral line syndrome. X 120

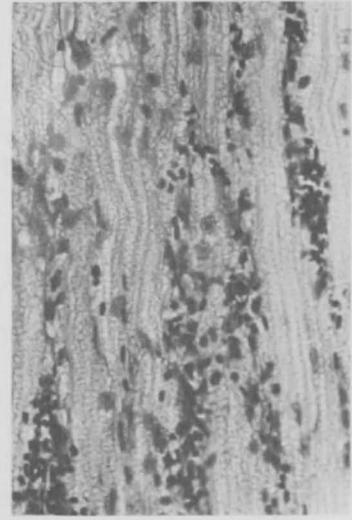


Figure 23:--Extensive vacuolation of muscle fiber protoplasm (sarcoplasm) in a severe case of chinook lateral line syndrome. Vacuoles are in continuous rows along all of the muscle fibers and blood cells fill the dilated blood vessels of the intermuscular fascia. X 460

Washington locality. Bunny Lake is at an elevation of more than 11,000 ft in the High Sierras of California. Results of the histological study to date indicate most tissues are within normal limits but gonads in Bunny Lake trout are generally underdeveloped or are undergoing degeneration and atrophy. It is evident that Bunny Lake trout do not spawn. This work, done in cooperation with Norman Reimers of Convict Creek Station has resulted in a review of 537 microslides to date.

#### Hemapoiesis

Progress in salmonid hematology in September was represented by results of a two and one-half day study session at the Western Fish Disease Laboratory during which those attending formulated appropriate detailed cytological descriptions for each cell stage in each series of developing blood cell types including the adult cells. Since September we have been standardizing staining techniques and photomicrographic techniques with a view toward future cooperative publication of an atlas of normal and pathological salmonid hematology illustrated in color. That a need for such a publication exists is recognized by many fishery biologists today. Professional advice and experience indicate that Eastman Kodachrome II is the film of choice for color photomicrography. However, since this film is not yet available in the indoor type an 80-B (bluish) filter must be used when exposing film.

#### Chinook lateral line syndrome (CHILLS)

This interesting purpuric or congestive disease of hatchery-raised chinook salmon fingerlings caused appreciable mortalities during the spring of 1963 at several nearby hatcheries. Morphological changes were characterized by slight reddening of skin areas around fin bases, lips, gill and opercular muscles, anal region and in particular the lateral line musculature. Stripping of skin from the lateral line region sometimes revealed a reddish-purple color which was most marked adjacent to the lateral line and faded progressively as the distance from the lateral line increased. The disease was at first believed to a hemorrhagic

disorder localized in the above-mentioned areas but microscopic study of stained sections magnified at one thousand diameters indicated a congestive condition accompanied by muscular degeneration which was most severe in those skeletal muscle fibers closest to the lateral line, figures 20, 21 and 22. The abundant accumulation of blood cells, almost entirely within capillaries surrounding muscle fibers and in minute vessels of the intermuscular fascia, was rich in immature red blood cells (rubricytes and metarubricytes) and many of the adult red cells contained shriveled nuclei. Some fish showed slight vacuolation of lateral line muscles but in one of the more severely affected ones these muscles were heavily vacuolated and largely degenerated, figure 23. Kidney smears for bacteria were negative after Gram staining. Unfortunately neither blood nor blood smears were taken from these fish. It is regrettable that, too often, in cases where moribund fish have been sampled for diagnosis of disease, insufficient material was taken to permit even a reasonably complete clinico-pathological study; the inevitable result being either an inaccurate diagnosis or none at all.

Thus far, in our study of "CHILLS", the histopathology suggests the presence of a toxic degenerative process possibly coupled with some form of anemia but an accurate diagnosis must await further study of the condition with emphasis on clinical pathology.

#### GENERAL

During late July and early August Dr. Halver conferred with a number of laboratory directors, research scientists, fishery managers and food plant managers in France, Italy, Germany and Southern England, prior to attending the Sixth International Congress of Nutrition in Edinburgh, Scotland, August 9 to 15.

Conferences were arranged on different specific subject matter with research scientists or institutes to discuss problems of mutual interest and to exchange recent research data: Professor Elaine LeBreton and staff at the Gustav Roussy Institute for Cellular Research in Paris, on dietary vectors in experimental animal liver

cancer; Professor Maurice Fontaine of the Museum of Natural History and Institute of Oceanography in Paris, on advanced fish physiology and biochemical mechanisms involved in thyroid metabolism and blood clotting mechanism; Professor Desnuelles, Professor of Biochemistry, University of Marseilles, on recent technological changes and effects in modern preparation of peanut oil fat, fat structure, fat alteration and types utilized by experimental animals; Professor Serra and Dr. Ghittion at the Fish Disease Center, Turin, Italy, on current advancements in Southern Europe on hepatomagenesis studies; Professor Liebmann and Dr. Kies of the Brauchtwasser Institute of Fishery Research in Munich, Germany, on fish husbandry techniques and physiological controls of metabolism; Dr. Andre Berg and others of the European Atomic Energy biological laboratories at Pallanza, Italy, on new methods for tracing biological systems and measuring biomasses in a water environment; Dr. Roe and others in the roundtable seminar at the Royal Cancer Institute, Chester Beatty Laboratories in London, on a review of chemical structure, stereo configuration, identifying techniques and physiological effects of aflatoxin in many experimental animals; Dr. Bampton and others at the Tropical Products Institute, London, on current techniques in the production of peanut, cottonseed, and soybean oil meals plus identification of mycotoxin contaminants; Dr. Ruth Allcroft in seminars at the Ministry of Agriculture and Fisheries, Weybridge, on impaired fat metabolism in hepatomagenesis; Dr. Pye Finch at the Scottish Home Department, Pitlochery, Scotland, on Scottish salmon husbandry problems; Dr. Don deLange of Pretoria, S.A. in Edinburgh, on use of new experimental animals in nutritional research; Dr. von Limbarg and co-workers of the Dutch Trout Industries on methods for early detection of hepatoma; Dr. Braekkan of the Government Vitamin Laboratories in Bergen, Norway, on new techniques for estimating vitamin availability, amino acid availability, and metabolic test systems for protein and fat availability studies; and Dr. Rollefson of the Bergen Aquarium and Institute for Marine Research on the design, and construction of modern aquarium and research laboratories using fish as experimental animals in physiology,

biochemistry, nutrition and other life science studies. Also included were conferences with feed plant managers and many managers of large hatcheries in southern France, northern Italy, southern Germany, southern England, Scotland and Norway.

In summary the time available was used to review current status of fish husbandry research, hepatomagenesis research and fish physiology with contemporaries in Europe, and to review current techniques in food science and technology used in the manufacture of fish and other animal rations in Europe. Papers were discussed at the Sixth International Congress of Nutrition on experimentally induced hepatoma in rainbow trout, and on the significance of circulating blood amino acids as an estimate of the amino acid requirements of the species. A number of informal planning sessions were arranged on needed future research to elucidate the specific vectors involved in the appearance of hepatoma in fish populations.

#### Construction and major equipment acquisition

No major construction was done during this year.

A porous stone, automatic recycling filter was installed in the well water supply for the experimental hatchery. Occurrences of aquatic insect larvae in the well water supply and kidney disease in some of the experimental lots of fish indicated a channelization from the Little White Salmon River to the well aquifer and necessitated this purchase. The filter will remove particulate matter greater than 12.7 microns in size, hence disease problems should be controlled. During installation of the filter all fish were removed and the experimental hatchery was sterilized. Hereafter the only fish used in the experimental hatchery will be hatched there.

Major equipment purchased during the past year included a well scintillation counter for the  $I^{125}$  isotope, and a Model 303 Atomic Absorption Spectrophotometer with accessory lamps for the determination of calcium, magnesium, sodium, potassium, copper, zinc and iron. This instrument should assist the inorganic



requirement and metabolism research. It allows greater speed, accuracy and sensitivity than classical wet methods of inorganic analyses.

A Zeiss research microscope with fluorescent microscopy attachments was also purchased.

#### STAFF

Dr. John E. Halver, Supv. Res. Chemist  
Dr. Gilles J. LaRoche, Chemist  
Dr. Bradford C. Croston, Chemist  
Mr. Arthur N. Woodall, Chemist  
Dr. Laurence M. Ashley, Fishery Biologist  
Mr. Warren E. Shanks, Chemist  
Mr. George D. Huestis, Administrative Asst.  
Mr. Robert R. Smith, Animal Husbandman  
Mr. George D. Gahimer, Fishery Technician  
Mr. Pete E. Benville, Chemist  
Mr. Clarence L. Johnson, Physiologist  
Mr. David F. Nash, Chemist  
Miss Martha J. Tripp, Chemist  
Mr. Charlie E. Smith, Fishery Biologist  
Mr. Max L. Larson, Fishery Aid  
Mrs. Myrna Morones, Clerk-Typist  
Mrs. Dana N. Eshleman, Histopathology  
Technician.

Mrs. Virginia L. Huestis, Scientific Secretary  
Mr. Montie C. Peterson, Fishery Aid  
Mrs. Hazel J. Jones, Physical Science Aid  
Mr. Albert E. Merritt, Fishery Aid  
Mr. Robert J. Knox, Student Trainee  
Mrs. Carlie M. Southard, Fishery Aid  
Mrs. Dale D. Odle, Clerk-Typist  
Mrs. Margie M. Hoover, Fishery Aid  
Mr. Bill P. Carter, Fishery Aid  
Mrs. Mary E. Cairns, Clerk-Typist  
Mrs. Caroline L. Perkins, Physical Science Aid  
Mrs. Bonnie F. Ternahan, Fishery Aid  
Mr. Danny L. Woods, Fishery Aid  
Mr. Gordon C. Baker, Maintenceman  
Mr. Walter Brost, Maintenceman Foreman  
Mr. Arthur C. Engel, Laborer

## HUSBANDRY METHODS

### CALIFORNIA-NEVADA SPORT FISHERY INVESTIGATIONS

Reno, Nevada

Reed S. Nielson, Chief



Convict Creek Experiment Station, Bishop, California

#### HIGHLIGHTS

The field phase of an intensive study of seasonal and interseasonal variations in aquatic organisms in Convict Creek together with an evaluation of the natural feeding propensities of stocked trout was completed. Eighty-five species of aquatic insects representing 54 genera of 36 families have been identified from 360 stream bottom samples.

Bunny Lake brook trout, now 13 years old, were sampled intensively for histological study and 10 living specimens were successfully transferred to the laboratory in October.

Streamside vegetation was shown to be the main source of particulate organic matter in Convict Creek. Fluctuations paralleled seasonal changes in volume of flow and short term changes were associated with windstorms, precipitation runoff, and the winter freeze-thaw phenomenon of the stream.

Dr. John A. Maciolek received an award from the Bureau in recognition of superior accomplishment in his preparation of Research Report 60 entitled, "Limnological Organic Analyses By Quantitative Dichromate Oxidation."

Construction of a 6-room addition to the laboratory building was completed in October.

#### SURVIVAL AND VITALITY OF HATCHERY-REARED RAINBOW TROUT, 1962-63

Experiments initiated in May 1962 in cooperation with the California Department of Fish and Game and the Western Fish Nutrition Laboratory were terminated May 28, 1963. Catchable-size, pellet fed, rainbow trout reared at four California hatcheries (Darrah Springs, Hot Creek, Moccasin Creek, and Moorehouse Springs) were used in these tests. The experiment was designed to investigate the role of hatchery water quality in relation to the quality of the trout produced as measured by their physiological adapt-

ability to a natural stream environment. Specimens were drawn from these populations at frequent intervals during the year, after stocking in Convict Creek, to determine changes in muscle and blood serum electrolytes, body chemistry (proximate analysis), and blood characteristics. Analysis of these samples is nearing completion by the cooperating Western Fish Nutrition Laboratory.

#### STREAM TEST OF MASSACHUSETTS BROWN TROUT

The first experimental stream survival trial of this hatchery strain of brown trout, which is just coming into use as an alternate to the catchable-sized rainbow trout for some regional management programs in California, was initiated in June. Three hundred of these pellet fed trout were held in a stream pen for a week, the usual pre-experimental observation period, without loss. Following the holding period, 250 were stocked in two in-line stream sections at a density of 133 pounds per acre. The remaining 50 were retained in the stream holding pen for subsequent observations under starvation conditions. These trout were not in prime physical condition when received, being distinguished by reduced or eroded caudal and pectoral fins, a susceptibility to fungus, and an average overweight condition. They had been retarded by underfeeding for a number of months and age at stocking was 18 months rather than the usual 10-12 months of age of rainbow trout used in all previous survival trials of hatchery fish. The trout were reared at Hot Creek Hatchery and were the only ones available in the catchable-size range for this experiment. On receipt, the weight index was 3.42 fish per pound. Initial measurements were made of total length, weight, condition factor (K), and three hematological indices (red cell count, hemoglobin, and hematocrit). These measurements were repeated on November 5, 1963, as a part of the pre-winter census. The data may be compared in the following summary:

Date	Length (cms.)	Weight (gms.)	Condition (K)	RBC	Hemo- globin	Hemato- crit
6/6/63	22.4(50)	132.5(50)	1.162(50)	1.45(10)	12.8(10)	41.8(10)
11/5/63	23.1(25)	110.9(25)	.887(25)	1.09(10)	10.3(10)	36.2(10)

Sample size in parentheses

Body condition (K) loss for the period was about average (24 percent) and blood quality declined about the same as has been experienced with various groups of rainbow trout in the past.

Survival for the period June 11 to November 3 (147 days) was 84.8 percent which is above average in consideration of elapsed time and superior to that of any of the rainbow trout groups for a comparable period in 1962. Known mortality since November has been negligible. Final checks of survival and health will be made in May 1964.

#### STREAM TEST AND SURVIVAL SELECTION OF RAINBOW BROOD TROUT

Three hundred selected fall-spawning brood rainbow trout, reared at Hot Creek Hatchery on fresh-frozen ocean fish and other wet-diet components to the catchable-size range (average 3.7 fish per pound) were stocked in two controlled experimental stream sections of Convict Creek on July 19, at a density of 147 pounds per acre. High seasonal runoff and the need for a free bypass channel necessitated the later stocking of this group. The stream survival phase of this experiment will be terminated in May, 1964. Initial and pre-winter measurements of body condition and blood quality are summarized on the next page.

In this group, condition loss was essentially the same as for the brown trout (23 percent) but blood quality did not decline. The reason or reasons for this can only be conjectured: wet diet rearing, three weeks shorter residence in the stream, or possibly better environmental conditions in the stream sections in which they were stocked. However, these apparent advantages over the brown trout were not demonstrated in their survival of only 79 percent.

At the conclusion of the stream test, selected survivors will be returned to the California Department of Fish and Game to be maintained

Date	Length (cms.)	Weight (gms.)	Condition (K)	RBC	Hemo- globin	Hemato- crit
7/19/63	22.0(50)	124.1(50)	1.150(50)	1.37(10)	12.2(10)	38.8(10)
11/5/63	22.9(25)	107.7(25)	.894(25)	1.29(10)	12.4(10)	38.8(10)

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at a hatchery for sexual development and experimental breeding to provide possible insights into the genetics of trout survival potentials.

#### STARVATION TESTS OF MASSACHUSETTS BROWN TROUT AND SELECT RAINBOW BROOD TROUT

Surplus brown and rainbow trout in the selected size range (same initial averages as the stream-stocked groups) were held in the stream-starvation enclosures for fall comparisons with the stream-stocked fish. Averages were as follows on November 6, 1963:

	Brown trout	Rainbow trout
Length (cms.)	23.0	22.0
Weight (gms.)	104.9	88.1
Condition (K)	.851	.822
Red Blood Count	1.2	1.16
Hemoglobin	11.0	10.1
Hematocrit	38.5	30.1

Somewhat greater weight and condition losses were experienced in the enclosure than in the open stream by both groups (see earlier tables). Blood quality changes, however, presented a curious picture in that brown trout indices remained slightly higher among the penned fish than for those in the stream. Rainbow trout blood became slightly (but insignificantly) poorer among the starved members than for those having the freedom of the stream. In both cases, differences between stream and starvation groups were insignificant but interesting in that they took different directions.

#### EXPERIMENTAL INTRODUCTION OF TROUT FOOD ORGANISMS

Efforts to establish introduced populations of the freshwater amphipod Gammarus lacustris were continued in 1963 with the release in selected locations of well over 1.5 million indi-

viduals collected (a) routinely from a prepared nursery area, and (b) fortuitously in heavily populated irrigation ditches that were drained for rechanneling. Five waters were stocked during the year: a small, acid-reaction lake; a small highly alkaline lake; two warm-spring-fed streams (60-65° F.) tributary to larger waters; and a 50-acre lake that supports a mixed-species trout population. Observations will be made to determine extent of success of the introductions as time and accessibility permit. Similar checks are scheduled for five additional waters not previously supporting amphipods which have been stocked with Gammarus since 1960. Of the ten waters stocked, positive indications of persistence have so far been observed in three. In one of these, Laurel Lake, stocked in 1959 and 1960, the organism occupies all bottom areas and is permanently established. No new introductions are planned unless more productive collecting areas for Gammarus can be found.

An introduction of about 200 crayfish (Astacus klamathensis), including many egg-bearing females, was made into another lake in October. This species was tested in the laboratory earlier in the year (February to April) and demonstrated cold-hardiness for adaptation to otherwise suitable alpine lakes. It is highly successful in Topaz Lake, a coldwater reservoir, where it is an important food item of fast-growing trout.

#### History of Gammarus introductions and resurvey of sierra waters involved in transplants

Inquiries and study of conservation club and fishery agency records, made this year, revealed that many alpine lakes of the Eastern Sierras were stocked with "feed" for trout at one time or another during the period of developing trout fisheries (1920-40). Most of these attempts to improve food conditions were made with Gammarus lacustris or a smaller relative Hyallela azteca, which could then be collected in quantity

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from relict sources such as vegetated springs, and a fair share of the introductions reputedly succeeded in improving trout food conditions. A list of 45 lakes has been compiled identifying sites of attempted introductions. A beginning has also been made on a survey of present day amphipod importance in those of the lakes for which the likelihood of endemic or naturally transferred populations is slight, to aid in better evaluation of the long-term success of the early introductions. Association of amphipod abundance and physico-chemical conditions is expected to give field criteria for environmental requirements. The goal is the establishment of practical limits to artificial distribution of the animal in oligotrophic alpine lakes.

#### AGED BROOK TROUT OF A HIGH ALPINE LAKE

##### Ecology and histology as related to advanced age

Food conditions, feeding, and trout growth were again measured at Bunny Lake during summer and fall months to determine possibly for the last time, whether the continuing reduction in trout numbers in this small lake (2.5 surface acres) has conferred any benefits upon the stunted trout survivors or their food supply. This year the volume of bottom organisms was too low to be accurately estimated from samples, (less than 0.1 cc/sq.ft.) and the average length of trout captured was 19.2 centimeters, as compared with 19.3 in 1962. A restoration of the invertebrate fauna seems unlikely as long as any trout remain in the lake.

A comprehensive series of gonadal and endocrine tissue samples was collected and forwarded to the collaborating Western Fish Nutrition Laboratory for histological processing and study, following conferences with Drs. L. M. Ashley and O. H. Robertson prior to the field season. The histology has confirmed that senile change is not advanced in glands, internal organs, and other tissues of these 13-year-old fish, although they have now lived more than twice the ordinary life-span of the species. However, some expected anomalies in reproductive tissue have been observed. Figure 1., Western Fish Nutrition Laboratory photo, depicts a malformed and hemorrhagic mature

testis in which the germinal cells - as in other mature testes of the group - reached the final spermatozoon stage in a non-functional condition.

##### Health and development under improved environmental conditions

Ten more of the aged brook trout from Bunny Lake are now being maintained in stream water at Convict Creek in addition to the single specimen reported on earlier as an example of normal growth resumption after 10 years of stunted existence. This group of 10 fish was back-packed from the lake in a rubberized pack sack without the use of drugs. They will be kept at the laboratory for a year, if possible, for observation of sexual development and resumption of growth. Of several attempts to hand-carry small numbers of these trout to the laboratory, only those made late in the season have had any success. Exploding bacterial infections, untreatable without great risk to such small (8-10) groups of fish, have resulted in 100 percent mortality within two weeks when the fish are transferred to water more than 10° F. higher than the lake.

Feeding of these excessively wild trout is less of a problem if more than two or three of them are held in the same trough. In the new group, fresh frozen brine shrimp are accepted but other smaller groups and individuals have taken nothing but live food consisting of scuds, earthworms, and immature aquatic insect forms as available.



Figure 1:--Abnormal testis of brook trout from Bunny Lake

## PRODUCTIVITY OF ALPINE WATERS

### Stream seston studies--particulate organic matter in convict creek

Routine measurement of the microscopic particulate organic content of Convict Creek within the station area was carried out between September 1962 and November 1963. Frequent samples were taken to observe the nature and quantity of organic particles in terms of daily and seasonal levels, to determine their source, and to ascertain the probable cause of any major fluctuations.

In the presence of macroscopic material, water samples were prefiltered through a 0.3 mm-mesh screen, then filtered through glass fiber pads to separate the microseston which was subsequently measured by quantitative oxidation. The year's observations are shown in figure 2. The plot of weekly means was smoothed somewhat by averages of 3 to emphasize the seasonal pattern.

Despite the low level of seston in the stream (averaging less than 1 mg per liter), significant fluctuations were observed. Usually, these corresponded to changes in stream flow. The figure 2 curve closely parallels observed stream flow patterns, highest seston content appearing at the spring runoff surge. Daily fluctuations were slight during warm weather and stable flow conditions but in mid winter a cycle was observed in which seston content doubled between early morning and midday. This corresponded with anchor ice formation and dissipation (i.e., daily flow cycle). Occasional ephemeral pulses in seston content occurred which were due to precipitational or aeolian causes.

In all instances, the seston was composed primarily of amorphous organic particles. This fact, together with the above observations, indicates that streamside vegetation is the main source of the sampled seston. It arrives in the stream through the actions of windstorms, precipitation runoff, and inundation by rising waters enhanced by the winter freeze-thaw phenomenon.

A study complementary to the foregoing was conducted on Laurel Creek to answer the questions of how and why the microseston content fluctuates downstream along the course of a small drainage system. The stream was selected because of the following advantageous ecological features:

1. It is a simple, pristine drainage traversing an altitude range of 2,500 feet in five miles.
2. It arises as a series of seepages and rivulets which drain a barren granitic area.
3. At the headwaters, it flows through one noteworthy and rather productive lake.
4. Two areas exist where it takes long precipitous plunges. The first is a 500-foot drop a short distance below the lake and the second is a 1,000-foot plunge down the terminal moraine at the base of the drainage.
5. Between the cataract sequences there are a series of relatively flat meadow lands (two miles), part grassy and part brushy (willow, alder, and aspen).

Eight water sampling stations were established at key points along the drainage and stream bottom samples were taken at these and various intermediate points. Water samples were taken in July, August, and October and treated as in the foregoing study of Convict Creek. Data from the final (October) strengthened earlier observations and conclusions presented in the September 30, 1963, Quarterly Progress Report. Important findings listed sequentially are:

1. Headwaters and spring seepages are organically poor containing only about 0.2 mg/liter of particulate organic matter.
2. Laurel Lake "charges" the stream with seston (about 2.5 mg/liter) in the form of effluent plankton (unicellular and small colonial algae).
3. Responding to the abundant food (limnoplankton), great masses of filter-feeding Simulium larvae appeared in the stream below the lake in

late summer. They effected the removal of about 40 percent of the seston within a quarter-mile section of stream.

4. During the stream's plunge over the first escarpment, most of the remaining limnoplankton is lost, presumably by mechanical disruption and chemical oxidation of the cellular matter. It is partly replaced by organic detritus from streamside vegetation.

5. A seston minimum occurs in the uppermost meadow due, in part, to the above (4) decimating factors and to dilution by seston-poor seepage waters which significantly increase stream flow volume.

6. Seston content increases as the stream passes through the meadow land. Nearly all of the gain consists of detrital organic particles.

7. Seston increases during final 1,000-foot plunge down the terminal moraine in contrast with the earlier loss under comparable flow conditions. Apparently the gain results from a combination of large detritus contributed by dense streamside vegetation and resistance of this type of seston to mechanical disruption and/or oxidation.

#### Productivity and illumination in some altitude lakes

The availability of photometric equipment and services of Mr. Robert W. Holmes of the Scripps Institution of Oceanography (Figs. 3 and 4), permitted evaluation of the light environments, relative to primary production, in a few lakes of the upper Convict Creek drainage. Samplings and measurements made during August included: lake seston content, primary production by periphyton and  $C^{14}$  techniques, vertical light attenuation (intensities) both upward and downward, and incident total light energy in selected shoal areas. Following are highlights of the data evaluated thus far:

1. Bottom-reflected light in shallow water: The bottoms of clear lakes can often be seen to considerable depth from the surface.

A logical conclusion is, therefore, that a significant amount of upward reflected light exists, at least under certain circumstances. We have previously suggested that this factor may play a measurable role in autotrophic production in lakes of this area.

Measurements were made with a standard underwater photocell screened with a green filter (Wratten No. 61). The cell was oriented upside down in "coffee-can" collimator which restricted its viewing angle to about  $60^\circ$  thereby reducing the influence of scattered light. It was necessary to amplify the cell's output because of the low intensities of prevailing light. Reflected light patterns were measured in direct sun during late morning hours at seven stations in two lakes where water depth ranged from 5.5 to 10.5 meters. All resulting curves of plotted data were quite similar. Three of the most extreme ones are shown in figure 5., with a deep water reference curve which represents scattered light only. The most striking feature of these shallow-water curves is that they have a similar slope -- steeper (less attenuation) than that of the scattered-light curve. Light intensity at a point 1 to 3 meters from the bottom either remains constant or increases slightly with depth. Positions of the curves along the horizontal axis (intensity) appear to be a function of time of day (solar altitude), depth of water above bottom and bottom reflectivity.

2. Shoal productivity and incident light energy: Although available light energy at mid-summer is not likely to limit autotrophic production in open water, it could possibly do so in less exposed shoal areas. This premise was tested by assessing the growth of periphyton at six shoreward stations in two lakes and comparing it with measurable factors of the respective light environments. The latter required mapping of sky exposures at shore-points adjacent to the periphyton sets, determining the relative amount of sunlight for each sky exposure, and primary measurement of the total available incident light energies (cf., Limnological Apparatus).

These data (table 1.) show no distinct relation between productivity and the indirect available light measurements of sky exposure



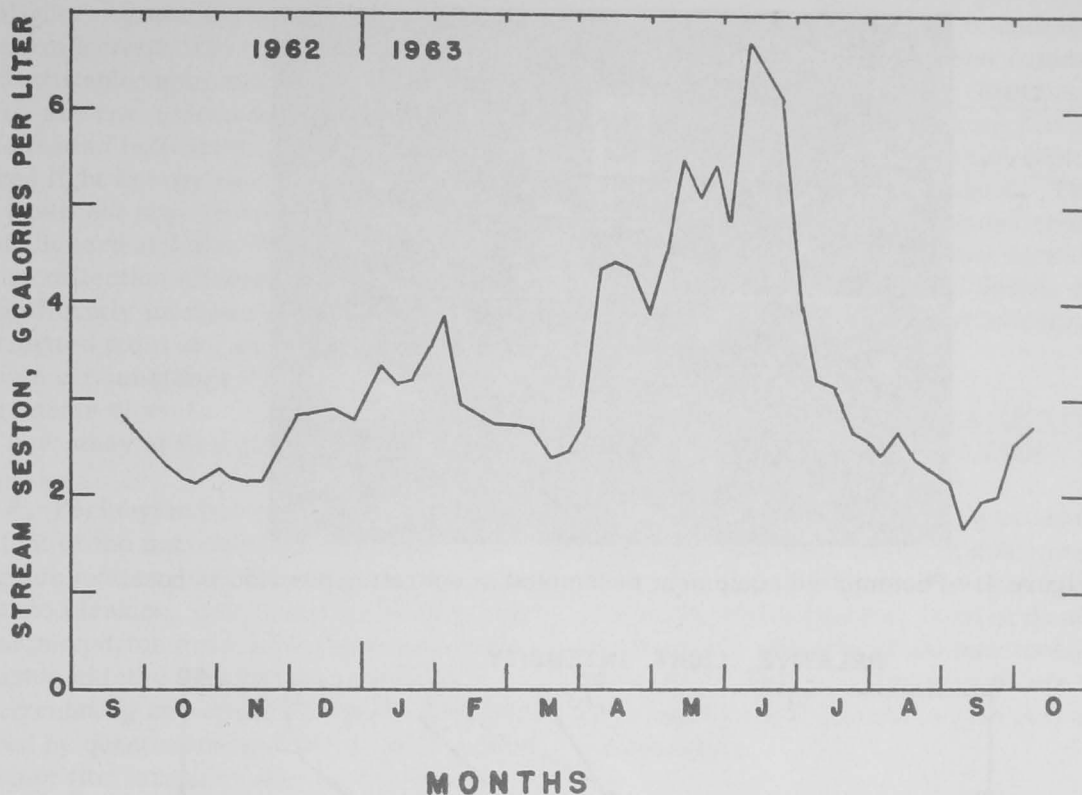


Figure 2:--Microseston content of Convict Creek at the Experiment Station for the year September 1962 to October 1963. One mg/liter organic weight approximately equals 4.7 gcal/liter.



Figure 3:--Mr. Robert W. Holmes, Scripps Institution of Oceanography, contemplates photo-metric equipment used for light measurements in Lakes Edith and Cloverleaf, August 1963. Gear shown includes: deck and sea cells (selenium barrier layer), null meter, damped Rawson microammeter, collimator can for reflected light measurements, Hewlett-Packard amplifier and associated power supply (12-volt storage batteries and sine-wave inverter).



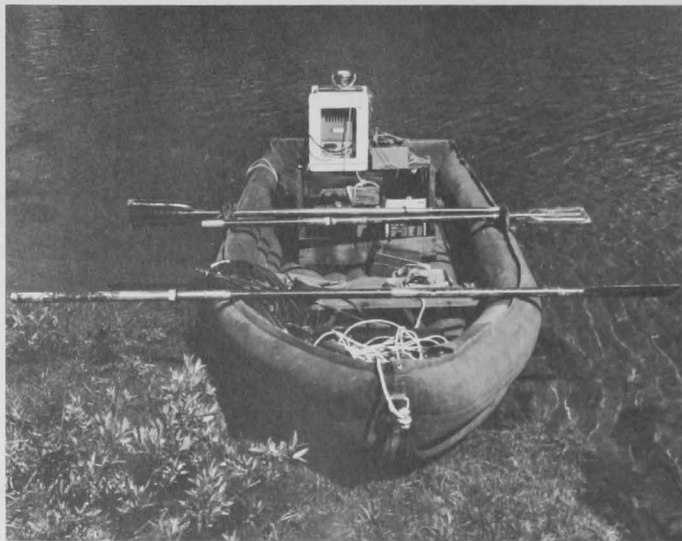


Figure 4:--Photometric equipment assembled in operating position in boat .

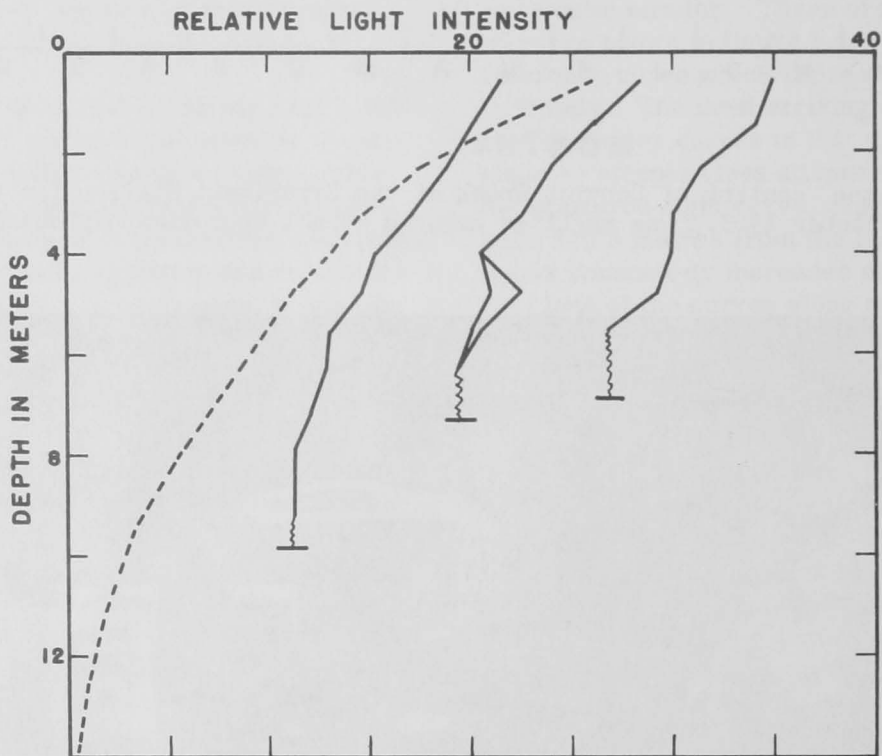


Figure 5:--Light intensities at four stations measured with a downward-facing photocell. Solid line curves were taken in shallow water (bottom indicated by horizontal bar) and contains a reflected light component. Dashed line curve is a scattered-light component. Dashed line curve is a scattered-light reference taken in deep water, about 30 meters.

and sunlight. Agreement does exist, however, between productivity and the direct measurement of available light energy, especially if the two lakes are considered separately. These observations also indicate an anomaly between measured light energy and its secondary estimation (percent sky + percent sun) in the west shoal of Cloverleaf Lake. It seems quite possible that reflection off surrounding terrain may significantly increase incident light over that estimated from sky and sun alone. If true, this single circumstance suggests that primary measurements of available light energy are essential in a study of this nature.

3. Periphyton production and the plankton content of the surrounding water: Particulate organic matter, representing mainly lacustrine nanno plankton, was measured before and after the periphyton growth period to see if any relationship existed between the quantities of freely circulating and attached algae. Both were measured by quantitative oxidation; the plankton from composite samples taken at 4 levels in the uppermost level of each lake, and the periphyton from sets placed at 1-meter intervals between the surface and a depth of 7 meters. Results are given in table 2. No relation between the two populations is evident among the six basins investigated.

#### LIMNOLOGICAL SURVEY OF CROWLEY LAKE

The limnological study of Crowley Lake was limited to the summer months and was confined to the accumulation of hydrographic data and the establishment of sample stations. The collection of routine data at these stations included: temperature, dissolved oxygen, transparency, conductivity, pH, plankton, benthos, and water. Aliquots of water samples were glass-fiber filtered in the laboratory for subsequent pigment analysis (chlorophyll a, b, and c and astacine and non-astacine carotenoids), and for dichromate oxidation of seston. Lugol's solution was added to other sample aliquots to preserve plankton for subsequent identification and enumeration. Weak thermal stratification was observed the latter part of May, dissipated in June because of protracted stormy weather, and reformed in early July. A well established thermocline was observed late in July and through

August. Highest epilimnion temperatures (67-69° F.) were attained in August coincident with a heavy algal bloom (mainly Gleotricha sp.). Serious oxygen deficiency (from 3.0 ppm to less than 1.0 ppm) in the hypolimnion (below 30 feet) was also observed during August. These phenomena occur annually and undoubtedly account for the poor quality of the trout as well as some mortality during the period. Secchi disc transparency was 13 feet in August as compared with 25 feet in May.

#### METHODS AND APPARATUS FOR LIMNOLOGICAL STUDIES

Three time-integrating actinometers consisting of photocell activated coulometers were assembled and field tested in conjunction with the productivity studies. Two of these performed well while the third functioned intermittently. Design improvements are needed to simplify the units and to gain greater ease of operation.

#### BIOLOGICAL EVALUATION OF EXPERIMENTAL STREAM SECTIONS

A two-year study of the densities and types of trout food organisms collected with the Surber Sampler from the four controlled stream sections of Convict Creek was completed on June 17, 1963. Volume and weight was determined for each group of organism in each of a total of 360 samples taken during the two-year study period. Identification was carried to the species level wherever possible. Principle groups present were: caddisflies, beetles, mayflies, stoneflies, dipterans, annelids, nematodes, water mites, snails and clams, and flatworms. To date 36 families representing 54 genera and 85 species have been identified. The Order Trichoptera is represented with the most species. A new species of roundworm of the family Mermithidae, Mesomermis genus was collected. This organism is of interest because it is occasionally parasitic on mayfly nymphs. Several unidentified aquatic invertebrates remain in the collection, especially among the Chironomidae, which must be reared artificially for reliable identification.

Table 1:--Light environment and autotrophic production for six shoal areas in two high altitude lakes.

Shoal station	Illumination Factors			Periphyton <sup>4/</sup> production <sup>4/</sup> (gcal/dm <sup>2</sup> )
	Percentage		Incident Light energy <sup>3/</sup> (gcal/cm <sup>2</sup> /day)	
	Sky <sup>1/</sup>	Sunlight <sup>2/</sup>		
Lake Edith: West	61	70		7.0
North	63	71	585	4.9
South	64	73	602	6.5
East	65	74		4.5
Cloverleaf Lake:				
Deep arm -East	65	79	617	12.3
Deep arm -West	73	81	580	6.8

<sup>1/</sup> Based on polar coordinate plot of horizon azimuths and elevations assuming 100% = sky area when horizon appears at 90° from vertical at all azimuths.

<sup>2/</sup> Portion of theoretically possible sunlight on a clear day (August 15) for this latitude as calculated from data in the Smithsonian Meteorological Tables.

<sup>3/</sup> Average values for clear days measured by barrier cell actinometers.

<sup>4/</sup> Upper 3 meters of water.

Table 2:--Average plankton content and 3-week periphyton production in top 6 meters of four alpine lakes, August 1963.

Lake	Plankton content (gcal/liter) <sup>1/</sup>	Periphyton production (gcal/dm <sup>2</sup> ) <sup>1/</sup>
Bunny	2.2	5.3
Cloverleaf:		
Inlet arm	1.6	23.1
Deep arm	3.1	16.9
Outlet arm	3.1	6.9
Edith	4.0	2.6
Genevieve	1.7	2.6

<sup>1/</sup> Divide values by 5 to obtain approximate ash-free dry weight in milligrams

Data on the volume of aquatic organisms indicate a remarkably reproducible order of production among the experimental stream sections. Greatest abundance was found in Stream Section IV with a standing crop (two-year average) of 155 pounds per acre followed in order by Section II, 133 pounds per acre, Section I, 113 pounds per acre, and Section III at 95 pounds per acre. Highest standing crop was observed during the period November through June with no noteworthy peaks of abundance occurring although production was somewhat greater during the second year of study.

Figures 6 and 7 compare the bottom fauna composition in percentages of total volume of all organisms for the two years. The data are grouped for the four stream sections. Three orders of aquatic invertebrates (Plecoptera, Trichoptera, and Oligochaeta) constituted 73 percent of the total volume in 1961-62 and 80 percent in 1962-63. The standing crop of bottom fauna was generally higher in 1962-63.

#### FOOD HABITS OF STREAM STOCKED HATCHERY-REARED RAINBOW TROUT

Rainbow trout reared at Darrah Springs, Hot Creek, Moccasin Creek, and Moorehouse Springs hatcheries of the California Department of Fish and Game were stocked separately in four experimental stream sections May 21, 1962. Stomachs for food analysis were collected from five trout from each of these hatchery groups at two-week intervals to November 6, 1962, and at monthly intervals thereafter to May 21, 1963, when this phase of the study was terminated. The stomachs were preserved in formalin and the qualitative and quantitative (volumetric and gravimetric) analysis of contents is in progress. The food consumption of the trout will be correlated with the abundance and types of invertebrate organisms found in the stream.

#### GENERAL

Visitors included the summer school class in Fisheries from the University of California (Berkeley), other student groups from the California Institute of Technology and Los Angeles State College, and Drs. M. Dale Arvey

and Edward Raney who were making a survey and study of biological field stations for the National Science Foundation.

Tentative arrangements were made with the Branch of Quality of Water, U.S. Geological Survey, for a cooperative study of trace elements in water, fish, plankton, and benthos in four lakes of our study area to begin July, 1964.

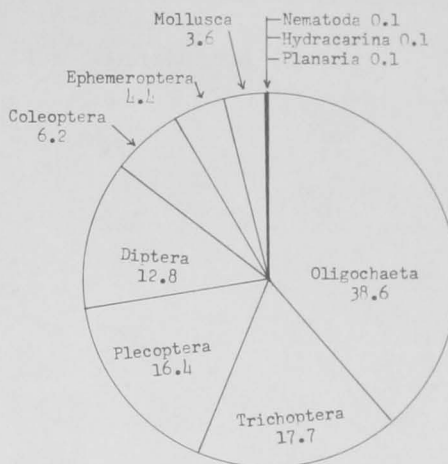


Figure 6:--Percent of total volume (cubic centimeters per square foot) of all organisms for the period July, 1961 through June 1962.

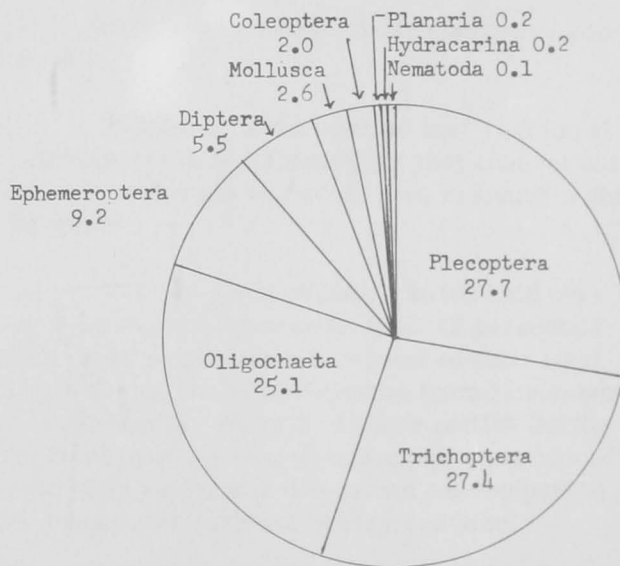


Figure 7:--Percent of total volume (cubic centimeters per square foot) of all organisms for the period July, 1962 through June, 1963.

## STAFF

Mr. Reed S. Nielson, Fishery Biologist

Mr. Norman Reimers, Fishery Biologist

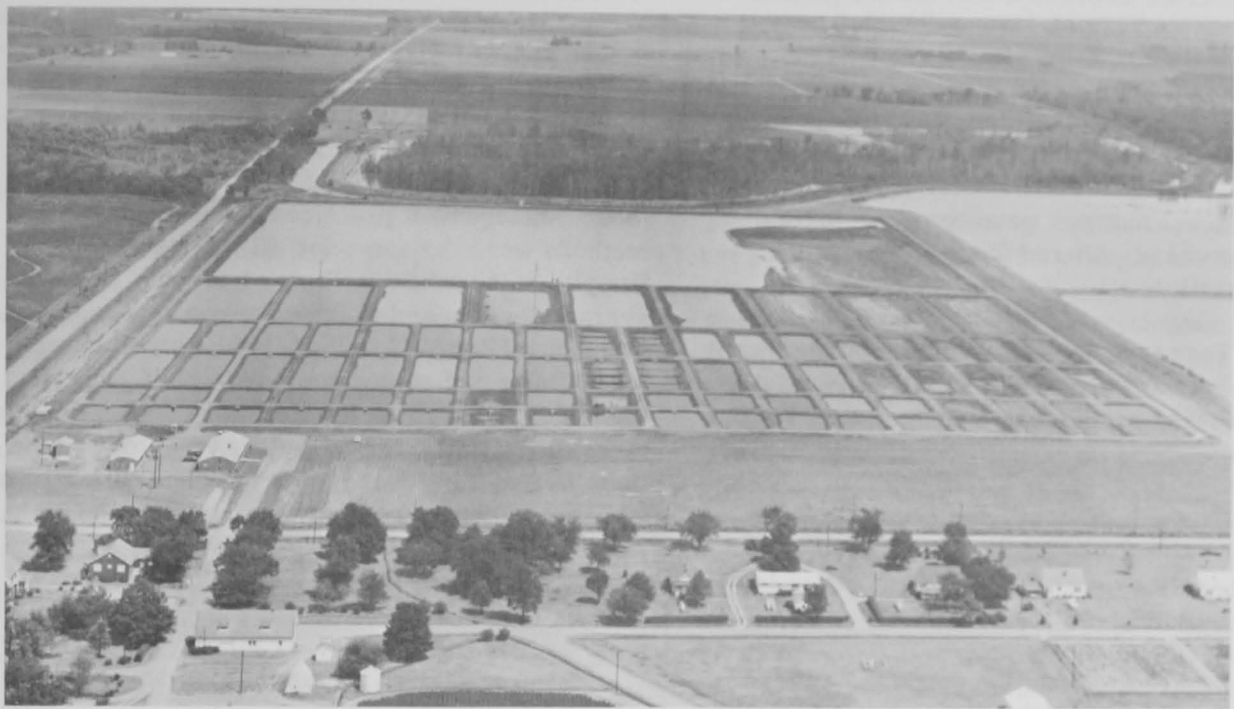
Dr. John A. Maciolek, Fishery Biologist

Mr. Harry D. Kennedy, Fishery Biologist

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



Fish Farming Experimental Station, Stuttgart, Arkansas

### HIGHLIGHTS

A channel catfish X blue catfish hybrid and three buffalo hybrids were produced at the station. These individuals have shown a more rapid growth rate than the young of the parent species.

Flathead catfish were successfully reared to fingerling size in troughs with a survival rate of 60 percent.

A stock of fingerling grass carp, *Ctenopharyngodon idellus*, has been received from Malaya for experimentation purposes.

Preliminary tests indicate that Korlan (Ronnel) may be an effective systemic control for *Lernaea* with only limited adverse effects on pond biota.

Claims concerning the effectiveness of Diquat for the control of Columnaris disease

could not be substantiated under controlled conditions.

Residue studies indicate that Guthion is concentrated in fish tissues but that channel catfish will eliminate or break down residues within six weeks.

Two-year-old channel catfish held over for a third growing season lost 12 percent of their body weight and 1.5 percent of their total length during the winter months from November through March. Growth of these catfish during the third growing season was about 1 percent of their body weight per day except for the period of gonadal development in May and June.

Channel catfish growth during their second year was at the rate of 2 percent body weight per day from April through July. After August 1, there was a decline in the rate of growth. Yearling fish gained 24 times their stocked weight, from 22 to 530 grams. Feed

conversion was about 1.4 and feed ingredient cost was about \$0.05 per pound of gain.

Fry of white, blue, and channel catfish grew at rates varying from 3.0 to 3.6 percent of body weight per day after stocking and achieved average weights ranging from 90 to 103 pounds per thousand. Feed conversion per unit of weight gain was slightly less than one.

Specifications were compiled for the manufacture of pelleted feeds and measuring pellet quality.

Pond fertilization studies indicate that zooplankton abundance is more closely related to the amount of nitrogen fertilizer added than to the amount of phosphate.

There is a significant correlation between the abundance of phyto- and zooplankton and the available nitrogen and phosphorus in the bottom soils of fertilized ponds.

Soil tests of station ponds indicate an increase in pH and in the calcium and magnesium content of the bottom soils.

Three pre-emergence type herbicides have given promising results for the control of rooted aquatic vegetation in station ponds.

An extension program is underway to assist fish farmers through contacts with County agricultural agents, visits with farmers and by the dissemination of information through newspapers and other media.

## FISH CULTURE

### Fish Hybridization

Hybridization studies resulted in the development of three crosses of buffalo fishes; black X bigmouth, black X smallmouth, and smallmouth X bigmouth\*. Fertilized eggs and subsequent fry were obtained by using hormone injections, hand-stripping, and artificial hatching methods.

Concurrent with the artificial propagation program, mixed pairs of breeders were injected

with chorionic gonadotropin and stocked in small ponds. One pair of the combinations successfully spawned, producing black X bigmouth hybrid offspring.

Comparative growth rate studies were initiated in tenth-acre ponds when the fry were 10 days old. Hybrids and nonhybrids (smallmouth and bigmouth) were stocked at the rate of 5,500 per acre and given the same type of feed. Management practices and sampling methods were the same for all groups of fish. Samples were taken at 20-day intervals and average lengths and weights were compared. The graph (figure 1) indicates the growth curve for each lot of fish involved. After 216 days, the black X bigmouth hybrids were approximately twice the length of the nonhybrids and four times their weight. No gross morphological differences were noted between any of the groups in the experiment.

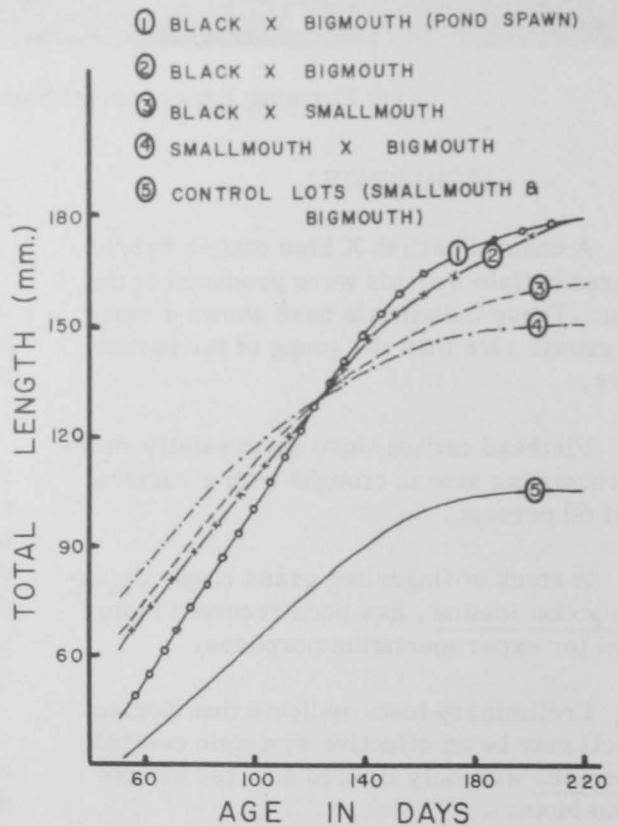


Figure 1:--Growth of hybrid and non-hybrid buffalo fingerlings

\*Female parent listed first in each cross.

A hybrid resulting from the cross of a female channel catfish with a male blue catfish was produced and its growth rate is being compared with the parent species. It has been observed that the rate of gain of the hybrid is 5.2 percent of its body weight per day while that of the channel and blue is 3.0 percent and 3.6 percent respectively.

### Flathead catfish

Flathead catfish fry were produced through hormonal stimulation of breeders in spawning pens and the eggs were hatched by mechanical means. Special emphasis was placed on the comparison of various starting diets and trough culture procedures. It was found that if raw fish containing thiaminase was used as food it should be supplemented with beef liver or thiamine hydrochloride.

Flathead fry were successfully reared to fingerling size in troughs with a survival of 60 percent. Complete mortality figures were recorded and all losses were attributed to specific causes. Two factors were responsible for 91 percent of the total loss. A suspected thiamine deficiency undoubtedly contributed to many of the fatalities attributed to parasitism and chemotherapy. Cannibalism was not considered a factor in the observed mortalities since no lacerations or teeth marks were evident on any of the moribund fish. No regurgitated or partially engulfed fish were seen. A summation of the above data was presented at the Seventeenth Annual Conference of the Southeastern Association of Game and Fish Commissioners.

Studies of growth rates, stocking and survival rates, and of species combinations concerning the flathead catfish are continuing. Yearlings, having been stocked in combination with buffalo, channel catfish, white catfish, largemouth bass, crappie, and Israeli carp for one season, demonstrated an average survival of 86 percent and attained an average weight of 1.5 pounds. When stocked alone at the rate of 50 per acre and provided with an abundance of forage fish, the average weight was 1.9 pounds and the survival rate was 92 percent.

### Introduction of the grass carp (*Ctenopharyngodon idellus*)

Among the various species of exotic fishes which have been proposed for use in fish farming reservoirs is the grass carp (*Ctenopharyngodon idellus*). This fish reportedly feeds extensively on rooted aquatic plants, grows rapidly, and has good eating qualities.

With the assistance of Dr. S. W. Ling, Fishery Biologist, FAO, arrangements were made to secure a stock of the grass carp from the Government of Malaysia. On November 16, 70 fingerling grass carp were received for experimentation purposes.

Upon arrival, the fish were given prophylactic treatments to prevent infection through handling and to avoid introducing foreign diseases or parasites. Following treatments, the fish were held under observation in two concrete holding tanks in the auxiliary building for one week. Thirty-two fish were then transferred to four large aquaria in the biology laboratory and the remainder were released in a tenth-acre pond. To date, eight fish of the indoor lot have died and it is believed this mortality was due to the difficulties encountered in inducing the fish to feed. Currently the fish are being fed a diet of canned spinach, a high protein fish meal, and earthworms. Two deaths have been observed in the pond fish. The remaining fish are active and feeding and appear to be adjusting to the new habitat.

### PARASITES AND DISEASES

#### Lernaea control

Pond trials of four organophosphates as feed additives for the control of *Lernaea* were concluded for the past growing season. Data from plankton collections, bottom samples, parasite indices, and from harvest figures were studied in an effort to evaluate the various chemicals. A summary of these data is presented in table 1.

Levels of parasitism were determined by recording the percent of individuals in a 200 fish sample which carried one or more parasites.

Table 1:--The effects of four insecticides fed to golden shiners in tenth-acre ponds, May - November, 1963.

PARAMETER	RUELENE 1000 ppm	CORAL 1000 ppm	DYLOX 2500 ppm	KORLAN 2500 ppm	CONTROL ---
Lbs. stocked per acre	200	200	200	200	200
Size when stocked	50/lb.	50/lb.	50/lb.	50/lb.	50/lb.
Fry survival	Poor	Fair	Fair	Excellent	Good
Lbs. per acre harvested	158.0	495.0	492.5	830.0	335.0
Size at harvest	52/lb.	67/lb.	51/lb.	205/lb.	147/lb.
Zooplankton					
Copepods	Eliminated	Not affected	Not affected	Not affected	-----
Cladocera	Eliminated	Eliminated	Eliminated	Eliminated	-----
Rotifers	Reduced	Not affected	Not affected	Not affected	-----
Bottom organisms	Reduced	Not affected	Not affected	Eliminated	-----
Parasite control	Fair except in July	Good except in July	Good except in July	Good through- out year	-----
Comments	To be discontinued	To try new formulation and increased level	To try new formulation and increased level	To try new formulation and increased level	-----

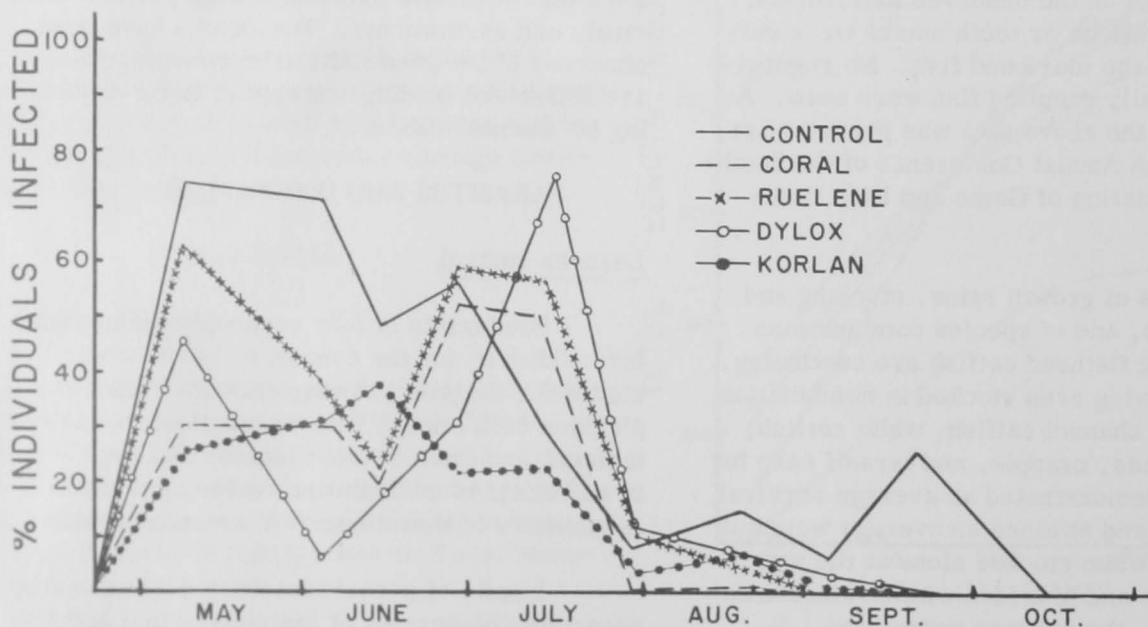


Figure 2:--Seasonal incidence of Lernaea

Plankton collections and checks of the parasitemia were made biweekly throughout the summer. Bottom samples were only taken monthly beginning in July. Earlier collections were not made since previous experience with other new ponds had indicated that several months are required for a bottom fauna to develop.

An examination of the harvest figures in table 1 indicates several relevant facts. The increase in poundage over the control in the Coral and Dylox ponds was due to larger fish rather than to an increase in numbers. Ruelene affected the survival of both the stocked fish and their fry as is indicated by the decrease in total poundage and average weight.

Although controlling effects were not complete, Korlan (Ronnel) was more effective than any of the other compounds. This chemical had only a limited effect on the fish. Survival of the stocked adults was high and a heavy spawn was produced. Production figures indicate that the population in the pond was approaching its capacity.

The reversal of controlling effects during July (second generation of parasites) in three of the ponds cannot be explained. Coral, Dylox, and Ruelene simultaneously failed to control the organism during this interval (figure 2) and, in all cases, the incidence exceeded that of the untreated lot.

Samples of minnow, vegetation, and mud from each pond were taken according to a prescribed schedule during the days following the cessation of feeding. These aliquots have been submitted to the chemical companies for residue studies.

Tissues from the liver, kidneys, and gonads have been collected from goldfish which received high levels of Coral, Dylox, Korlan, and Ruelene for a six month period. These tissues are now being processed into microslides for study in an effort to determine if pathological changes may be related to the pesticides. Fish from the same groups have been submitted to the respective chemical companies for residue analyses.

## Controls for Columnaris disease

Preliminary studies were made concerning the effectiveness of Diquat as a treatment for Columnaris disease. Representatives of the chemical company had indicated that this chemical was an effective control for the disease, even when applied in very low levels. Following the diagnosis of the disease in a large population of golden shiners at a local fish farm, applications of Diquat were made at various levels in the laboratory. Evaluation of the results, however, was difficult since some of the fish exhibited only cutaneous lesions, others harbored systemic infections, and still others had both. Using the presence of moving cells as an indication of life, the following results were obtained using 25 infected fish in 15 gallon aquaria: (+ indicates the presence of live organisms)

<u>Concentration</u>	<u>4 hrs</u>	<u>8 hrs</u>	<u>22 hrs</u>
50 ppm	+	-	-
25 ppm	+	+	-
12.5 ppm	+	+	-*
6.25 ppm	+	+	-
1.0 ppm	+	+	+
Untreated	+	+	+

\*)Although no organisms were present in the external lesions, live cells were found within an eyeball lesion.

Following these results, further tests were conducted in 30 cubic foot tanks at the fish farm using large quantities of minnows of various sizes as they were being processed. Rates of application included 6.25, 12.5, 25.0, and 50.0 ppm for a 48 hour period. All lots of treated fish appeared healthy and behaved normally. The incidence of external lesions rose sharply, however, in the untreated groups. The untreated groups of minnows were then subdivided equally for further testing. Although there was a difference in the rate of loss, all lots of fish were in very poor condition. Fish in all groups continued to die, many without ever exhibiting lesions. Systemic infections were easily demonstrated from moribund fish and these were considered to be the cause of death.

It thus appears that Diquat may inhibit subacute infections if only cutaneous lesions are



involved. However, most fish exhibiting external lesions quickly develop a septicemia. Such infections are not controlled by applications of Diquat up to 50.0 ppm.

#### Pseudomonas infections

A manuscript on the description and treatment of a Pseudomonas infection in white catfish has been completed and submitted to the Journal of Applied Microbiology for consideration leading to publication.

#### Scoliosis and lordosis in catfish

Skeletons of deformed channel catfish were submitted to Dr. I. V. Ponsetti of the Department of Orthopedic Surgery at the University of Iowa Medical School. After examining the skeletons, Dr. Ponsetti suggested that the scoliosis and lordosis may be linked in some way to pathological changes in the pineal body. Studies are now underway to check this hypothesis.

#### Diagnostic services

Diagnostic services were provided throughout the year by staff personnel at the laboratory or during field visits. Outbreaks of Columnaris disease were numerous in the Stuttgart area but did not appear to affect minnow producers in other areas of the State.

Requests for assistance were received from several State agencies. A fish kill affecting gizzard shad and largemouth bass in Lake Maumelle near Little Rock, Arkansas was diagnosed as Columnaris disease. At the request of the Louisiana Pollution Control Commission, Dr. Meyer visited the Mississippi delta to observe a major fish kill that was in progress. Numerous bacterial isolates were made but these failed to indicate a single organism as the lethal agent. No organism was found which was common to all species of fish involved in the kill and it thus appeared that if bacteria were involved in the mortality, they were probably secondary.

#### Feed processing

After an initial purchase of 1,000 pounds each of six test rations, we acquired a 10 h.p. hammermill with several screen sizes, a 10 cu. ft. mixer, platform scales and a California Pellet Mill and installed them in a local soybean extraction plant. Pellets can be made in 1/8", 1/4" and 1/2" diameters using four die speeds and variable-speed feed intake. Steam, water and liquid mixtures can be added to rations by batches or continuously.

Early season research on feed acceptability and availability resulted in the adoption of the following processing specifications:

1. One-eighth inch diameter die operated at 305 RPM.
2. Pellet cut-off length of 1/16" for fry; pellets of 1/4" in length for larger fish, increasing to 7/8" as the season progressed.
3. Steam, 6 percent of feed weight, added during pelleting, removed during pellet cooling.
4. Fiber content of formulas, 8 to 9 percent minimum.
5. Oil or water additives limited to 1 percent, except in formulas containing 10 percent or more of rice hulls, in which case 3 percent water was added during mixing and 3 percent steam during pelleting.

Specifications were also compiled for measuring pellet quality, this being, in effect, a measure of stability in water. Ten grams of test rations were 90% retained on a No. 14 mesh screen after 10 minutes immersion in water. High (19 percent) fiber pellets were 97 percent retained in a standard laboratory test and 50 percent recovered from a pond bottom after 30 minutes.

#### Growth and feed conversion

Three-year-old fish were fed at the rate of 2 percent calculated weight, second-year fish

at 3 percent body weight and young-of-the year at 5 percent. Monthly sampling permitted corrections in the projected weight curves used to adjust feed requirements on a daily basis. During the 210-day season, fish were fed 188 days.

1961-class channel catfish - catfish yearlings were stocked in June 1962, in sixteen quarter-acre ponds at the rate of 1,500 per acre. Eight rations were tested in duplicate during the 140-day season. At the November harvest, 93.8 percent survival was obtained.

Feed formulation is shown in table 2. Ration 1 was a commercial feed bought from warehouse stock. Ration 2 was the "Auburn No. 2" formula. Rations 3 and 4 were designed to test the replacement value of poultry by-product for fish meal. Rations 5, 6, 7, and 8 replaced fish meal with increasing amounts of soybean meal.

An evaluation of these rations in terms of fish growth, feed conversion, cost of feed per pound of gain and weight gain per acre, are shown in table 3. Rations containing fish meal gave better results than those with none. Rations 5, 6, and 7 were above average in production and below average in cost. These three rations and Ration 1 were retained for a third-year growth test. Formulation of Ration 1 was modified at the start of 1963 to include fish meal and distillers solubles, replacing poultry by-product meal.

Survival for the original sixteen ponds on April 1, 1963, was 93.3 percent. Wintering loss in weight averaged 27 grams and in length 0.43 centimeters. At the end of the 1963 season (November 1) survival was 85.7 percent which included two ponds having a 20 percent kill due to oxygen depletion. With the fish lost directly to low oxygen figured into the total population at the survival rate for all other ponds, the adjusted survival for three years was 90 percent.

Growth, survival and condition factors for channel catfish during the third year are shown in table 4. Weight gains and feed conversion are found in table 5. The largest fish and best gains were fed Ration 5, as in 1962.

Yearlings - Channel and white catfish were stocked into quarter-acre ponds at the rates of 1,500 and 2,000 per acre to study maximum growth on "least-cost" formulas. Formulas 9 and 10, (table 6) are modifications of rations selected by an electronic computer satisfying eight requirements considered essential for a supplemental feed. Formula 9 allowed a maximum fiber of 6 percent; formula 10 allowed 10 percent fiber. Data for second-year growth are summarized in tables 7 and 8.

Channel catfish stocked at 1,500 per acre gained in weight from 22 grams to 530 grams, and in length from 12 to 36 centimeters. No significant difference was found between the two rations. Daily weight gain was 2 percent per day for four months, then decreased for a seasonal rate of 1.6 percent. Weight gains per acre averaged over 1,500 pounds. Survival for four test ponds and a control pond was 95.1 percent.

White catfish gained less than channel catfish during the season. At higher stocking rates, less weight was attained by each species, but higher pond yields resulted. Poor feed conversion for white catfish stocked at 1,500 per acre may have been caused by over-feeding. These fish did not gain as did the channel catfish, although the same feeding schedule was maintained.

Fry - During the summer, catfish fry were stocked as available from federal hatcheries. Stocking rate was about 12,000 per acre. Table 9 presents growth and feed conversion data of these fish. Channel catfish grew better than whites and these in turn better than blues. Survival was 98 percent for all fry stocked. Feed conversion was 0.8 to 1.0 in the four ponds tested.

Fry stocked in August received a feed similar to Ration 9 plus vitamins and minerals. No advantage was observed in feeding a complete ration over a supplemental ration.

Table 2:- Ingredients, in lbs. per ton, used in 1962-63 test rations.

Ingredients	Ration							
	1	2	3	4	5	6	7	8
Rice bran	600				200	200	200	200
Yellow corn meal	50				200	150	100	50
Wheat shorts	50				400	400	400	400
Dried whey	100				200	200	200	200
Soybean meal	300	700	1000	1000	400	650	900	1150
Cottonseed meal	600		400	400				
Distillers solubles		300	300	300				
Poultry by-product	200			300				
Fish meal		300	300		600	400	200	
Peanut oil meal		700						
Salt	40							
Trace minerals	1							
Dicalcium phosphate	40							
TM-10	4							
Vitamin A (10,000/g)	4½							
Vitamin D <sub>2</sub> (41500 ICG/g)	4½							
Vitamin B <sub>12</sub> (25 mgms/lb)	1							
Cost*	\$66.80	78.00	81.80	77.40	80.80	77.50	74.20	67.60

\* Ingredient prices based on August, 1963, market quotations in FEEDSTUFFS.

Table 3:--Growth and feed conversion of yearling channel catfish during the 140-day 1962 season (Average of duplicate ponds).

Ration	Number Per Acre		Harvested Average		Conversion	Feed Cost Per lb. Gain	Weight Gain lbs. Per Acre
	Stocked	Harvested	Weight grams	Length cms.			
1	1500	1426	207	28.2	1.81	\$0.062	620
2	1500	1370	223	28.7	1.77	\$0.069	643
3	1500	1446	256	30.2	1.44	\$0.059	815
4	1500	1331	204	28.1	2.26	\$0.087	598
5	1500	1430	295	31.7	1.40	\$0.056	838
6	1500	1392	282	30.8	1.35	\$0.052	865
7	1500	1412	273	30.7	1.37	\$0.051	849
8	1500	1420	225	28.6	1.66	\$0.056	704

Table 4:--Growth of channel catfish during the third growing season (average of duplicate ponds).

Ration	Number Per Acre		Stocked		Harvested		1)	2)	Condition Factor (K)
	Stocked	Harvested	Weight grams	Length cms.	Weight grams	Length cms.	$R_w$	$R_l$	
1	1426	1252*	191	28.2	891	43.3	0.7%	0.2%	1.09
5	1394	1290*	260	31.2	974	43.3	0.6%	0.15%	1.20
6	1390	1384	250	30.9	945	44.1	0.7%	0.17%	1.15
7	1380	1298	245	30.6	851	43.0	0.6%	0.16%	1.05

\* A 20% kill occurred in one duplicate pond due to oxygen depletion. Conversion data adjusted to include all weight gains of known dead fish.

1)  $R_w$  = rate of weight gain per day as found by the equation  $W_n = W_0 \left(1 + \frac{R}{100}\right)^n$

2)  $R_l$  = rate of length gain per day

Table 5:--Weight gains and feed conversion of channel catfish during the third year (average of duplicates).

<u>Ration</u>	<u>Average Gain</u> grams	<u>Conversion</u>	<u>Feed Cost</u> Per lbs. Gain	<u>Weight Gain</u> lbs. Per Acre	<u>% Survival</u> 1962-63 Overall
1	700	2.46	\$0.091	1935	83*
5	714	2.37	\$0.095	2020	86*
6	695	2.21	\$0.086	2120	93
7	606	2.23	\$0.094	1740	86.5

\* 20% kill occurred in one duplicate pond due to oxygen depletion.

Table 6:--Ingredients, in lbs. per ton, used in low-cost 1963 test rations.

<u>Ingredients</u>	<u>Ration</u>	
	9	10
Soybean meal	300	300
Cottonseed meal	200	200
Alfalfa meal	80	80
Ground rice hulls		200
Rice mill dust	100	200
Rice chits and weed seeds	400	300
Rice polish	200	200
Rice bran	200	
Fish meal	200	200
Meat scraps	200	200
Feather meal	100	100
Salt	20	20
Cost*	\$64.85	\$60.74

\* Ingredient prices based on August, 1963, market quotations in FEEDSTUFFS.



Table 7:--Growth of yearling catfish, 1963 (average of duplicate ponds).

Ration	Species	Stocking Rate No. Per Acre	Stocked		Harvested		1)		2) Condition	
			Weight grams	Length cms	Weight grams	Length cms	$R_w$	$R_l$	Factor (K)	
9	Channel	1500	22	12	523	36.4	1.55	0.5	1.09	
10	Channel	1500	21	12	512	36.3	1.55	0.53	1.07	
9	Channel	1500	14	10	430	34.8	1.65	0.60	1.02	
Control	Channel	1500	20	12	80	22.1	0.7	0.20	0.74	
9	Channel	2000	22.7	12.7	416	34.5	1.50	0.50	1.01	
9	White	2000	36	18	366	30.2	1.60	0.30	1.33	
9	White	1500	23	13	381	30.2	1.30	0.40	1.37	

1)  $R_w$  = rate of weight gain per day as found by the equation  $W_n = W_0 (1 + \frac{R}{100})^n$

2)  $R_l$  = rate of length gain per day

Table 8:--Growth gains and feed conversion of catfish during their second year (average of duplicate ponds).

Ration	Species	Stocking Number Per Acre	Harvest Number Per Acre	Average Gain grams	Conversion	Feed Cost Per lbs. Gain	Weight Gain Per lbs. Per Acre
9	Channel	1500	1408	501	1.42	\$0.046	1554
10	Channel	1500	1431	491	1.39	\$0.042	1549
9	Channel	1500*	1148	416	1.86	\$0.060	1041
Control	Channel	1500	1288	60	-	-	172
9	Channel	2000	2000	395	1.53	\$0.049	1740
9	White	2000	1948	330	1.41	\$0.045	1420
9	White	1500	1495	361	2.29	\$0.074	1179

\* One-acre pond with an oxygen depletion killing 300 fish September 1. Conversion adjusted to include weight gains of all known dead fish.

Table 9:--Growth and feed conversion of young-of-the-year catfish.

Species	Date	Stocking		Date	Harvest		1)		Conversion	% Survival
		Weight grams	Length cms		Weight grams	Length cms	$R_w$	$R_l$		
Blue	6/28	0.5	2	10/30	38.6	16.5	3.6	1.7	1.0	99
White	6/28	1.2	4	10/30	45.4	15.8	3.0	1.1	0.9	95
Channel	7/18	2.7	6	10/30	46.7	19.0	2.8	1.1	0.8	96
Channel	8/19	5.4	9	10/30	46.0	18.8	3.0	1.7	0.9	99

1)  $R_w$  = rate of weight gain per day as found by the equation  $W_n = W_0 (1 + \frac{R}{100})^n$

2)  $R_l$  = rate of length gain per day

## Effect of enzymes, hormones, and anti-fertility compounds

Chemicals for anti-fertility work were available too late in the season for satisfactory testing. Single ponds were used for measuring the effects of proteolytic and diastatic enzymes and thyroprotein on growth. It was learned in September that the pelleting process as used in the laboratory destroyed about 90 percent of the activity of enzyme additives. Growth improvement was noted in the pond receiving thyroprotein. These materials will be tested again with replicate ponds before reporting on their value. A method for pelleting was devised to obtain a good quality pellet without using heat.

## LIMNOLOGY

### Nitrogen and phosphorus fertilization

Pond fertilization studies were continued from last year. Last year's data indicated that both nitrogen and phosphorus were necessary to produce and maintain desirable plankton blooms. Various combinations of these nutrients were used this year in 8 tenth-acre ponds in order to arrive at their most effective ration and amount.

Four ponds received 4 pounds of nitrogen per acre per application and 4, 8, 12, and 16 pounds of  $P_2O_5$  respectively. Four other ponds received twice the above amounts of nutrients per application, and all ponds received 8 applications with the exception of pond 8. This pond was treated with one part per million Simazine in June and a bloom could not be attained even with two additional applications of fertilizer.

Ponds 1, 2, 3, and 4 each received a total of 64 pounds of nitrogen and 64, 128, 192, and 256 pounds of  $P_2O_5$  per acre respectively. Ponds 5, 6, and 7 received 32 pounds of nitrogen and 32, 64, and 96 pounds of  $P_2O_5$  per acre. Pond 8 received 40 pounds of nitrogen and 160 pounds of  $P_2O_5$ .

Biweekly chemical and plankton analyses were made. From the results it is evident that, in the station ponds, nitrogen is needed at the rate of 8 pounds and  $P_2O_5$  at the rate of 8 to 12

pounds per acre per application. Eight to 10 applications were necessary in the ponds studied to achieve desirable results.

Zooplankton populations were higher in the ponds that received the higher rates of nitrogen (figures 3 and 4).

Slight differences in fish growth were apparent in the two sets of ponds. All ponds were stocked at the rate of 100 bigmouth buffalo and 50 channel catfish per acre. In the ponds fertilized at the higher rates, the average gain in weight of buffalo was 2.3 pounds, and for catfish, 1.2 pounds. The average gain of each in the other ponds was 2.1 and 0.8 pounds respectively.

There were only slight differences in chemical properties of all ponds (table 10). The amounts of nitrogen and phosphorus detected by chemical analyses did not reflect the amounts that were added to the ponds.

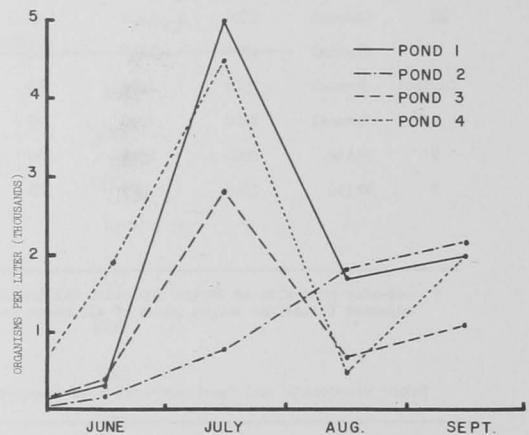


Figure 3:--Zooplankton abundance

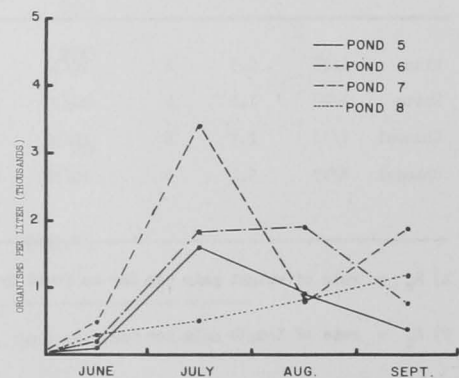


Figure 4:--Zooplankton abundance

Table 10: June - September averages of chemical analyses of study ponds.

Pond No.	1	2	3	4	5	6	7	8
Temp. °F	84	84	84	84	84	84	84	84
pH	8.6	9.1	9.0	9.3	9.3	8.8	8.9	8.9
O <sub>2</sub> (ppm)	6.0	8.4	10.0	11.6	8.7	5.9	9.1	8.6
Ph-th alk. (ppm)	14	32	25	41	37	17	19	27
M.O. alk. (ppm)	225	206	240	201	193	214	216	261
NH <sub>3</sub> -N (ppm)	.041	.024	.017	.018	.021	.023	.018	.012
NO <sub>3</sub> -N (ppm)	.11	.10	.10	.13	.12	.11	.10	.12
O-PO <sub>4</sub> (ppm)	.48	.68	1.05	.90	.31	1.23	.86	1.98
Total PO <sub>4</sub> (ppm)	2.7	1.2	3.4	2.5	1.3	2.8	3.2	2.0

### Soil, water, and plankton relationships

Eight tenth-acre ponds, each fertilized with different rates of commercial fertilizer, were used in a preliminary investigation of soil, water and plankton relationships. Soil, water and plankton samples were collected prior to each fertilization and analyzed for various chemical constituents and number and types of plankton. Table 11 shows, by correlation coefficients, the relationships that exist between the number of phytoplankton and certain water properties.

The data were separated into five components for the coefficient calculations. The column headed "overall" represents 42 pairs of observations and includes all the data. The "early" column has only 14 pairs and represents that portion of the data recorded soon after the fertilization program was begun. The "late" column includes 28 pairs and is representative of the data from the latter two-thirds of the program. Four of the ponds received 4 pounds of nitrogen per application and four received 8 pounds of nitrogen per application; the last two columns represent these groups and contain 18 and 24 pairs of observations, respectively.

These data indicate: 1) A highly significant direct correlation between the number of phytoplankton and the amount of ammonia nitro-

gen present in the water at the time of collection. This relationship is associated with high rates of nitrogen application and is present during the latter stages of the fertilization program. 2) The ortho-phosphate of the water and the number of phytoplankton are inversely related during the later stages of fertilization. 3) The numbers of zooplankton and phytoplankton are closely related late in the fertilization program and at all levels of nitrogen applications. 4) During the fertilization program, the phytoplankton far outnumbered the zooplankton and, at high rates of nitrogen and late in the program, the phytoplankton contribute more to the total volume of plankton than does the zooplankton.

Table 12 shows the relationship of zooplankton with the same pond properties as listed with the phytoplankton. The close relationship of zooplankton and phytoplankton populations can be recognized. Additional relationships exist with the content of nitrate and nitrite nitrogen at the time of collection. These relationships exist only during the early stages of the fertilization program.

Relationships existed between the available nitrogen in the soil and: the overall effect of the ammonia nitrogen of the water; the total and poly-phosphate of the water during the early period of fertilization; and the nitrate and ammonia nitrogen of the water at the lower rates of fertilization.

The available phosphorus in the soil was related to the ortho- and total phosphorus in the water during the early period of fertilization.

### Soil tests of station ponds

In 1962, after construction and prior to water impoundment, the bottom soils of all ponds were sampled. The pond bottoms were again sampled in June, 1963, after flooding. The soil test values are given in table 13. All analyses were performed by the University of Arkansas, Soil Testing Laboratory, Fayetteville, Arkansas. There is a wide variation in values between the ponds, as indicated by the large standard errors, which resulted from an inadequate sampling technique. A sampling method is needed that will obtain a known volume

Table 11:--Relationships, expressed as correlation coefficients, between numbers of phytoplankton and certain water properties.

	Overall	Early	Late	4 lbs. N Per Acre	8 lbs. N Per Acre
Secchi Disc	-.24	-.30	-.15	-.29	-.19
Nitrate Nitrogen	-.10	-.11	-.24	.17	-.33
Nitrite Nitrogen	.10	.02	.24	.26	-.02
Ammonia Nitrogen	.51**	.49	.51**	.27	.71**
Ortho-phosphate	-.22	.20	-.38*	-.23	-.21
Number of Zooplankton	.86**	.14	.88**	.96**	.77**
Total Plankton	1.00**	.60*	1.00**	1.00**	1.00**
Volume of Plankton	.61**	.36	.61**	.14	.88**

\* significant at the 5 percent level.

\*\* significant at the 1 percent level.

Table 12:--Relationships, expressed as correlation coefficients, between numbers of zooplankton and certain water properties.

	Overall	Early	Late	4# N Per Acre	8# N Per Acre
Secchi Disc	-.45**	-.24	-.36	-.47	-.42
Nitrate Nitrogen	.04	.75**	-.24	.31	-.23
Nitrite Nitrogen	.25	.86**	.32	.35	.17
Ammonia Nitrogen	.46**	.22	.39*	.38	.53**
Ortho-phosphate	-.12	.04	-.27	-.16	-.07
Number of Phytoplankton	.86**	.14	.88**	.96**	.77**
Total Plankton	.89**	.87**	.91**	.97**	.82**
Volume of Plankton	.50**	.42	.48**	.30	.66**

\* significant at the 5 percent level

\*\* significant at the 1 percent level

Table 13:--Mean soil test values at preimpoundment, 1962, and in June, 1963, of ponds at the Fish Farming Experimental Station.

Soil Tests	Preimpoundment, 1962	June, 1963
pH	5.15 ± 0.03*	7.17 ± 0.59
Organic Matter (%)	1.12 ± 0.04	1.02 ± 0.18
P <sub>2</sub> O <sub>5</sub> (lbs./acre)	12 ± 1	46 ± 21
K (lbs./acre)	189 ± 8	330 ± 16
Ca (lbs./acre)	2349 ± 96	4618 ± 1962
Mg (lbs./acre)	712 ± 42	1584 ± 275
Na (lbs./acre)	494 ± 31	319 ± 14

\* standard error of the mean.

of soil from flooded ponds. Work is currently under way to improve the sampling technique.

Between collection dates, there was an increase in the pH and the calcium and magnesium content and a decrease in the amount of sodium.

The increase or decrease in the soil test values due to the use of well or surface water is tabulated in table 14. Data from forty ponds, twenty flooded predominately with well water and twenty with surface water, were used in the calculations. Well water tends to deposit more calcium in the bottom soil.

#### Physical, chemical, and biological properties of station ponds

The collection of physical, chemical, and biological data from the station ponds was continued throughout this year. Recording of climatological data was initiated during the year. These data are being tabulated and placed in the station files for reference use.

#### POND MANAGEMENT

##### Species combinations and stocking rates

In early summer of 1962, four newly-constructed one-acre ponds were selected to

Table 14:--Increase (+) or decrease (-) of soil test values from preimpoundment, 1962, to June, 1963, resulting from the use of well or surface water on ponds at the Fish Farming Experimental Station.

Soil Tests	Well Water	Surface Water
pH	+1.64 ± 1.09*	+1.74 ± 0.59
Organic Matter (%)	+0.11 ± 0.05	+0.00 ± 0.20
P <sub>2</sub> O <sub>5</sub> (lbs./acre)	+33 ± 17	+42 ± 21
K (lbs./acre)	+144 ± 67	+148 ± 56
Ca (lbs./acre)	+4333 ± 862	+1864 ± 1329
Mg (lbs./acre)	+624 ± 343	+1044 ± 349
Na (lbs./acre)	-249 ± 205	-60 ± 111

\* standard error of the mean.

study growth rates of two stocking combinations of bigmouth buffalo, channel catfish and largemouth bass using either well or surface water. Two ponds were filled with well water and two ponds were supplied with surface water taken from a storage reservoir that had been filled with water from an adjoining bayou.

On June 11th, one surface-water pond and one well-water pond were stocked with 50 yearling buffalo, 75 yearling channel catfish and 50 bass. The other ponds were stocked with 100 yearling buffalo, 100 channel catfish and 50 bass. Ten pounds of fathead minnows were introduced into each pond to provide available forage for the bass.

Each pond was given an initial application of 80 pounds of 14-14-14 commercial fertilizer and subsequent weekly applications of 40 pounds of 15-30-0 fertilizer for 11 weeks during the summer of 1962. A total of 420 pounds of 15-30-0 fertilizer was added at intervals during the spring and summer of 1963. Monthly water analyses and plankton and benthos enumerations were made to correlate fish growth with possible differences in water quality and pond fertility. The fish population was sampled periodically and the ponds were drained November 14, 1963, when all fish were measured and weighed.



Table 15:--Comparative growth rates of three species of fish in one-acre fertilized ponds supplied with either well or surface water. Yearling fish were stocked June 11, 1962.\*

Pond	Species	Stocking Rate	Average Size After 5 Months		Average Size After 17 Months		Total Wt. (lbs.)
			Wt. (lbs.)	Length (In.)	Wt. (lbs.)	Length (In.)	
E 1 (Surface Water)	Buffalo	50	1.35	11.0	2.70	15.8	121.5
	Channel Catfish	75	0.31	8.0	0.78	14.4	54.6
	Largemouth Bass	50	0.42	9.5	0.90	12.0	<u>40.5</u> 216.6
E 4 (Well Water)	Buffalo	50	1.85	11.6	4.26	18.4	183.2
	Channel Catfish	75	0.36	9.0	1.20	15.7	81.6
	Largemouth Bass	50	0.38	9.0	0.72	11.5	<u>27.4</u> 292.2
E 3 (Surface Water)	Buffalo	100	1.48	11.1	2.67	15.9	251.0
	Channel Catfish	100	0.32	8.1	0.77	14.4	70.8
	Largemouth Bass	50	0.48	10.0	0.91	12.2	<u>40.0</u> 361.8
E 2 (Well Water)	Buffalo	100	1.88	11.8	4.31	18.3	379.3
	Channel Catfish	100	0.47	9.0	1.26	15.6	108.4
	Largemouth Bass	50	0.40	9.5	0.75	11.6	<u>30.8</u> 518.5

\* Stocking Size: Buffalo 6 = 1 lb. Channel Catfish 20 = 1 lb. Largemouth Bass 12 = 1 lb.

Table 15 shows average size of the fish at the end of the first and second summers. Since we were primarily interested in average size of the fish at the end of a two year rearing period, the gain in weight of each species is not shown.

The greatest growth of buffalo and channel catfish occurred in the ponds supplied with well water. The two stocking rates had little effect on the final size of the fish. Maximum average sizes of 4.31 pounds for the buffalo and 1.26 pounds for the channel catfish were found in Pond E 2, supplied with well water and stocked at the rate of 100 buffalo and 100 channel catfish per acre. Bass grew slightly faster in ponds receiving surface water, however, there was considerable range in the size of individual bass in all ponds.

At present an examination of the total plankton and bottom organism populations fails to reveal a significant correlation between the density of these populations and the larger size of the buffalo and channel catfish in the well-water ponds. Further studies may show signifi-

cant differences in the species composition of the basic food organisms of the ponds.

Differences in water quality of the ponds were the most apparent. Table 16 shows partial data collected from water analyses made in August, 1963.

The well-water ponds have a high dissolved mineral content as evidenced by methyl orange alkalinity, total dissolved solids and specific conductance values. Total calcium and magnesium ions show higher concentration in the well-water than in the surface-water ponds. Further studies and pond replicates may provide needed information to show a relation between the properties of water used at this station and fish growth.

In two one-acre ponds filled with well water, fertilized and stocked with a combination of species on May 16, 1963, there was relatively rapid growth of buffalo (table 17). After 6 months, at a stocking rate of 125 buffalo to the acre, the buffalo averaged 2.54 pounds and 2.23 pounds respectively. White catfish were stocked in Pond

Table 16:--Water analyses of four ponds (August 19, 1963).  
(Analyses in parts per million, except as indicated)

	Pond E 1 (Surface Water)	Pond E 2 (Well Water)	Pond E 3 (Surface Water)	Pond E 4 (Well Water)
Temperature (Degree F) 1 Ft.	82.0	83.0	82.0	82.0
pH (Units)	8.3	8.0	8.4	8.1
Alkalinity (M.O.)	92.0	188.0	88.0	180.0
Calcium (Ca ions)	12.0	127.0	12.0	120.0
Magnesium (Mg ions)	5.1	41.0	6.0	40.0
Nitrates	0.16	0.20	0.24	0.15
Ortho-phosphates	0.18	0.23	0.37	0.21
Turbidity	62.0	60.0	74.0	20.0
Sp. Cond. (Micromhos)	290.0	570.0	300.0	610.0
Total Dissolved Solids	174.0	590.0	186.0	626.0

E-7 in place of the channel catfish used in Pond E-5 to compare the growth rates of these species. The channel catfish reached an average size of 0.54 pounds while the white catfish grew to 0.43 pound.

Another pond, stocked at a higher rate, showed slower individual growth although the total pond production was approximately the same as that in Pond E-7. Studies of growth rates of these species in combinations will be continued through a second growing season to determine an optimum stocking rate for maximum individual growth and for maximum pond yield.

### Chemical weed control

Three recently developed pre-emergence type herbicides that have shown activity against a broad spectrum of weeds were selected for tests of effectiveness in the control of rooted aquatic vegetation. These included Casoran, Fenac, and SD 7961 (Shell). Preliminary tests

Table 17:--Growth rates of fish stocked in combination in fertilized one-acre ponds supplied with well water. Yearling fish were stocked May 16, 1963.

Pond	Species	Stocking Rate	Average Size Stocked		Average Size After 6 Months		Total Wt. (lbs.)
			Wt. (lbs.)	Length (In.)	Wt. (lbs.)	Length (In.)	
E 5 (Well Water)	Buffalo	125	0.40	9.0	2.54	15.5	284.5
	Channel Catfish	100	0.05	5.0	0.54	12.3	49.7
	Crappie	100	0.04	4.0	0.31	7.6	26.7
	Flathead Catfish	25	0.10	7.0	1.53	15.7	24.5
	Israeli Carp	5	0.30	8.0	4.34	18.4	21.7
	Fathead Minnows	10#	-	-	-	-	0.0
E 7 (Well Water)	Buffalo	125	0.40	9.0	2.23	14.9	254.2
	White Catfish	100	0.08	7.0	0.43	10.2	40.4
	Crappie	100	0.04	4.0	0.42	8.7	34.9
	Flathead Catfish	25	0.10	7.0	1.27	14.5	25.4
	Israeli Carp	5	0.30	8.0	4.70	18.8	23.5
	Fathead Minnows	10#	-	-	-	-	60.0

on the effects of these chemicals on benthic organisms were conducted in large aquaria. Results indicated that dragon fly naiads, midge larvae, snails, and crustaceans were not affected. Control of rooted aquatic plants was excellent, but filamentous algae became highly abundant.

The bottoms of 3 tenth-acre ponds were plowed, harrowed, and then divided into four equal quadrants. Each chemical was evenly distributed over one of the areas of each pond and the remaining area in each pond was left untreated. After application, the plots were hand raked to incorporate the material lightly with the soil and the ponds were then filled with well water. We hoped this arrangement would allow a comparison of the effectiveness of the three chemicals against each other and against the untreated area. It is clearly evident, however, that one or all of the chemicals were dissolved and transported over the entire pond. No rooted plant growth was evident in any of the treated ponds. Apparently the concentration of these chemicals in the water was effective in preventing weed growth in the soil of the untreated area. Filamentous algae occurred in all ponds.

A sufficient supply of Casoron was available to study it separately in a tenth-acre pond. In this case, the material was applied to the freshly drained pond bottom and the pond was immediately refilled. This was done to determine if incorporation into the soil was necessary for effective weed control. The results of this test indicate that the material is just as effective when applied on the surface of the bottom mud. No rooted aquatic weeds were observed in this pond during the entire summer.

Additional tests are planned which will allow the use of one chemical per pond. Different rates of treatment are also indicated since entire ponds were controlled by treating three-fourths of the bottom.

#### Biological weed control

Israeli carp were added to 12 station ponds to observe their effectiveness in aquatic weed control. Stocking rates were 15, 20, and

50 fish per acre and the fish were introduced into ponds with various combinations of other species of fish. Troublesome amounts of vegetation were not experienced in any of the ponds during the entire growing season.

One group of 8 ponds, 4 containing Israeli carp and 4 without, were used for one experiment. Filamentous algae was present in all but became a problem only in ponds without carp.

#### Fish barriers

Tests were completed on the use of Saran material for fish barriers. The material proved to be effective in preventing the passage of all sizes of fish into reservoirs. Mimeographed bulletins describing the uses and fabrication methods were prepared for distribution to interested fish farmers.

#### Agronomic research in fish-rice rotations

A major problem associated with the production of rice on reservoir soils has been excessive vegetative growth and subsequent lodging of the grain. Field observations and limited experimental data indicate that lodging is less likely to be a problem on reservoir soils of silt loam texture than on reservoir soils of clay texture.

Analyses of soil samples from reservoir and "old" rice field soils revealed that available nitrogen accumulated during the years of water submergence or fish production and was sufficient to be a major cause of lodging of the rice crop grown the following year.

An experimental area was selected within a reservoir with a clay soil of high available nitrogen content to secure information on the feasibility of growing corn, grain sorghum or soybeans to reduce the accumulated available nitrogen. All crop growth reduced the available nitrogen content from 175 pounds to approximately 40 pounds per acre. Growth of soybeans or other arable crops in the crops in the rotation program would thus appear feasible as a means for reducing the excessive available nitrogen content of reservoir soils.

Table 18:--Gross Guthion residues (ppm) following one application of one part per million (active) as wettable powder in a 0.1 acre pond.

Material	July* 1	July 2	July 3	July 5	July 15	Aug. 12
Control lots-flesh	< 0.1					
Control lots-viscera	< 0.1					
Green sunfish-flesh	1.9	***				
Green sunfish-viscera	4.6	***				
Golden shiners (whole)	5.1	***				
Gizzard shad (whole)	11.6	***				
Buffalo flesh	4.1	***				
Buffalo viscera	15.4	***				
Carp flesh	3.5	4.9	5.0	***		
Carp viscera	5.0	12.5	12.2	***		
Goldfish (whole)	5.8	9.5	6.9	***		
Channel catfish flesh	8.9	9.6	10.8	4.1	0.2	n.d.
Channel catfish viscera	10.2	24.0	18.4	4.6	0.3	n.d.
Plankton & pond water	0.8	0.5	0.4	0.1	n.d.	n.d.

\* Four hours after application.  
 \*\*\* No survivors from preceding day.  
 n.d. Not detectable.

The data obtained from the residue studies indicate that fish killed by Guthion are not fit for consumption. Catfish taken from freshly treated ponds would not be edible for at least two weeks but would contain no residues by the end of six weeks if not exposed a second time.

Collections of plankton and bottom organisms were taken from both the treated and untreated ponds. The abundance of the various organisms is presented in table 19. Only the number of nauplii seemed to be reduced following the application of the insecticide. However, the increasing number of adult copepods would indicate otherwise in spite of the fact that nauplii did not appear in the collections. Insect larvae (especially *Enallagma* sp.) were abundant in the pond ten days after the application was made and were found in every subsequent collection.

As predicted, channel catfish were the only survivors when the pond was drained in late October. Growth of the stocked fish was excellent. Fingerling catfish averaging one ounce were stocked at the rate of 500 per acre and supplied supplemental feed in the form of pellets during the experiment. When harvested, the fish weighed approximately one pound. Survival of the stocked catfish was good (90 percent) and no deformed fish were observed.

A fish-rice rotation test was initiated in July, 1962 at the Fish Farming Experimental Station to investigate the interrelations of fish and rice production. A series of station ponds was subdivided into four plots each through the use of polyethylene sheeting to provide experimental units. Over a period of four years, the plots will be rotated according to the following program: Fish, water without fish, rice, and rice with fish. Grain yields of Northrose rice, during the past year, from plots following the growth of fish or following water submergence without fish were not appreciably different. Data from a sufficient number of years have not as yet been collected for significant comparisons between rotations. However, there are changes in the soil analyses before and after growing fish, possibly caused by flooding of the soil, which appear to be real. The ammonia nitrogen increased at the 1-2 and 2-3 inch depths of soil but decreased at the 0-1 inch soil depth; ammonia nitrogen produced during incubation increased at the 0-1 inch depth and decreased at the 1-2 and 2-3 inch depths; and nitrate nitrogen decreased at the 0-1 inch depth but increased slightly at the 1-2 inch and 2-3 inch depths.

### Selective toxicants

Previous studies have indicated that Guthion is tolerated in fairly high concentrations by channel catfish but that other species of fish are highly susceptible. This property has led to the investigation of its potential use in controlling fish populations. During the past year, data have been collected concerning residues in fish and also on the effects of the application of Guthion on plankton and bottom organisms. These data were analyzed during this quarter.

One part per million Guthion (active) was applied as a 25% Wettable Powder in a tenth-acre pond stocked with a variety of fishes. Although the primary interest concerned residues in catfish, other species were collected as they became moribund and were also submitted for gross residue analyses. Plankton was concentrated with a net and frozen in pond water for similar analyses. Residues obtained from these materials are presented in table 18.

## Chemical control of tadpoles

TFM ( 3-Trifluoromethyl-4-nitrophenol) applied at the rates of 12 and 14 ppm effectively removed bullfrog tadpoles from treated ponds . Analyses of data obtained from collections of plankton and bottom organisms from these ponds indicate that the food organisms were not affected by either of the applications .

### GENERAL

#### Extension services

Mayo Martin, Fishery Biologist, was employed in September to develop an active extension program to facilitate the dissemination of research findings to the fish farmers . Emphasis to date has been placed on making contacts with fish growers to discuss their operations and to provide technical assistance . Mr. Martin has prepared several newspaper articles and has appeared before interested groups to discuss the various aspects of fish farming and fish culture .

Exhibits were prepared for the dedication of the Greer's Ferry Dam, Heber Springs, Arkansas, on October 3; for the Arkansas County Fair, DeWitt, Arkansas, September 18-21; and for the 28th Annual World s Championship Duck Calling Contest and Agricultural Festival, Stuttgart, Arkansas, December 4-7, 1963 .

#### Other

The steady flow of visitors to the station continued throughout the year . Interested visitors included fish producers, representatives from twenty foreign countries, twenty-four States and six colleges and universities .

#### Construction

Construction of water control structures to the unfinished ponds was completed and acceptance made on May 28 . The original complement of 81 ponds planned for the Stuttgart Station is now in use .

Table 19: - - Abundance of food organisms in Guthion treated (upper line) and untreated (lower line) ponds.

Date	Nauplii*	Copepods*	Cladocera*	Rotifers*	Total Bottom Organisms**
May 29	1160	100	45	1350	----
	230	0	0	15	----
June 21	85	10	15	10,550	----
	635	15	20	1,110	---- **
July 1 (Pretreatment)	0	65	440	8,100	----
	350	0	0	600	---- **
July 2 (24 hrs. post-treatment)	0	5	0	6,800	----
	320	0	0	610	----
July 3 (48 hrs. post-treatment)	0	5	10	2,305	----
	270	0	0	490	----
July 10	0	20	0	1,365	----
	5	0	0	45	----
July 26	0	30	5	245	520
	20	0	0	30	6
August 19	0	1,440	130	2,380	82
	0	110	90	200	22
September 10	0	415	0	815	18
	0	25	0	25	90

\* Organisms per liter.

\*\* Organisms per square foot. In the treated ponds, the predominant organisms were Tendipes, most of which emerged in late August; in the untreated pond Chaoborus was most numerous. Its increase in numbers is expected in late summer.





Figure 5:--A shipment of grass carp from Malaysia arrived at the station, November 16.



Figure 6:--Approximately 2,300 channel catfish ranging in size from one to three pounds were transferred to the Arkansas Game and Fish Commission upon termination of a number of research units.

## STAFF

Dr. James H. Stevenson, Fishery Biologist

Dr. Fred P. Meyer, Fishery Biologist

Dr. Waldon H. Hastings, Physiologist

Mr. James Mayo Martin, Fishery Biologist

Mr. John J. Giudice, Fishery Biologist

Mr. Joe B. Sills, Chemist

Mr. Dewey Lee Tackett, Chemist

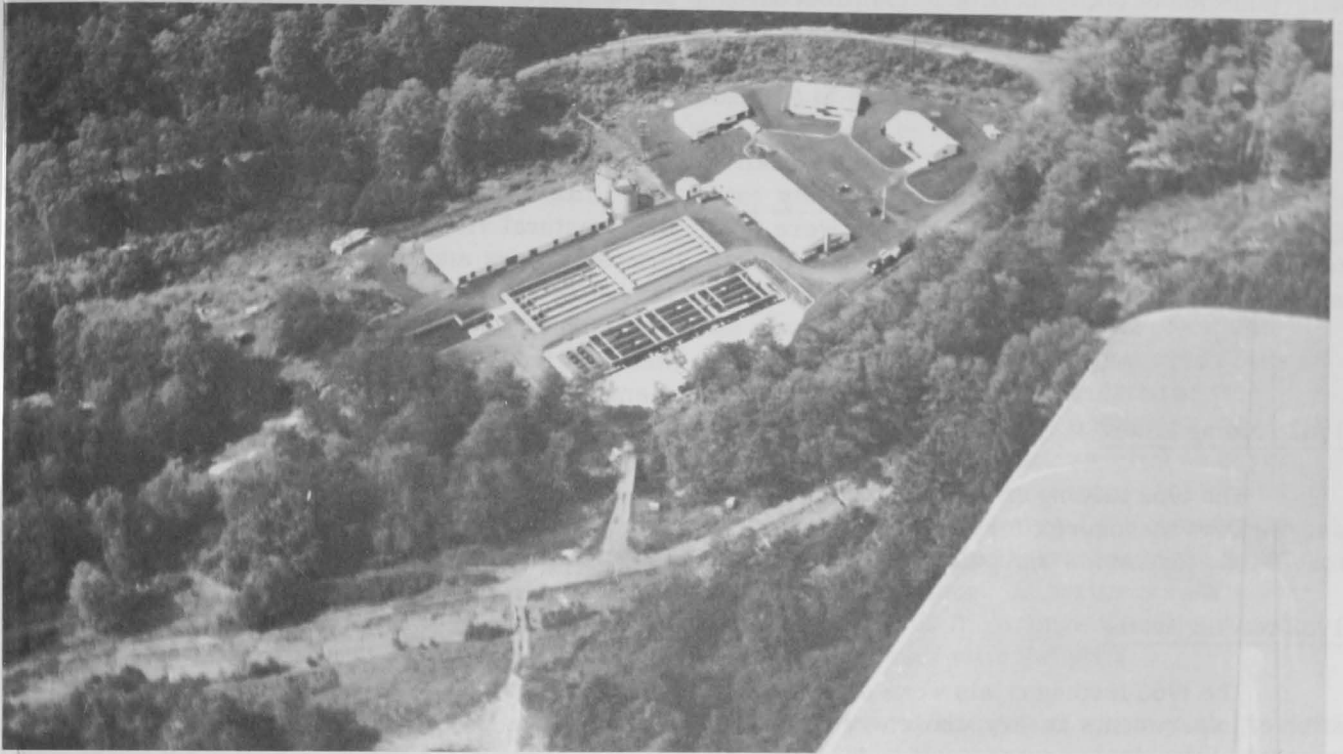
Miss Doris F. Allen, Clerk-Stenographer

Mr. Don S. Godwin, Fishery Aid

Mr. Ronnie L. Jarman, Fishery Aid

Mr. David A. Estes, Maintenceman

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



Salmon-Cultural Laboratory, Longview, Washington

#### HIGHLIGHTS

An extensively tested, reconstituted, all-meal diet has proved superior to standard production diets for the rearing of fall chinook salmon.

Larger fish of the same age have a higher oxygen demand indicating a higher metabolic rate. Conversely, older, larger chinook salmon fingerlings have a lower oxygen requirement per pound than younger, smaller fish. The rate of reduction changes abruptly at an average weight of five grams.

Chinook fingerlings held in aerated, recirculating water appear to tolerate gradual increases of ammonia for as long as 17 days.

Chemical differences in the blood plasma can be correlated with differences in fish-cultural procedures.

The percentage of small blood cells in hatchery samples of chinook salmon is inversely correlated with the performance of the fish in the stamina tunnel.

Chinook fingerlings normally respond to stressing by an increase in the percentage of small blood cells.

Fish up to five grams in weight show a progressive increase in swimming ability unaffected by environment. Above five grams, conditioning affects performance.

To date, sex control measures have proved ineffective.

An improved design in the cercarial electrocution grid has proved more economical and more efficient in the destruction of cercariae in the hatchery water supply.

The Salmon-Cultural Laboratory has assumed responsibility for operation of the Abernathy Incubation Channel. Preliminary to the determination of optimum production procedures, efforts are being concentrated on the development of a significant natural run of chum salmon.

More than sixteen tons of fingerling fall chinook salmon were liberated in Abernathy Creek and in excess of 1,000,000 eggs were taken from the adult chinooks returning to the station.

## NUTRITION

### 1962 feeding trials

The 1962 feeding trials were completed and the final manuscript forwarded to Washington, D. C. for review and publication.

### 1963 feeding trials

The 1963 feeding trials were a continuation of experiments at this laboratory for the purpose of developing nutritionally adequate diets for the artificial propagation of salmon. The objectives of the experimentation were to determine the optimum caloric levels in an all-meal diet and to determine the amount of crystalline vitamin supplementation necessary for adequate maintenance.

The composite meal, basal ration consisted of 35 percent salmon meal, 30 percent dried skim milk, 20 percent cottonseed meal, and 15 percent wheat germ, supplemented with four levels of crystalline vitamins ranging in amounts from 100 percent to 200 percent of the maximum levels recommended by Halver. The protein level was controlled by the addition of water to provide bulk in the diet. Caloric intakes ranged from 1,650 calories to 3,050 calories per kilogram of diet. Increases in the caloric levels were accomplished by substituting peanut oil for a portion of the water. All caloric levels were calculated on the basis of available calories as developed by Phillips.

All diets were fed to duplicate tanks of

fall chinook salmon fingerlings, bound by the addition of CMC, and ricer fed. The amount fed was allocated to the individual lots on the basis of body weight and water temperature.

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

1. Preliminary investigations indicated that a natural vitamin supplement consisting of brewer's yeast and liver meal, when added to the basal composite meal, produced fish with symptoms of vitamin deficiencies after four weeks of feeding. A crystalline vitamin supplement was necessary to prevent avitaminosis.
2. The growth rates of fish from all of the experimental diets were better than the all-meal control diet with the exception of the diets fed at the caloric level of 1,650 and the diet in which soybean oil meal was used as a replacement for cottonseed meal. Mortalities were significantly higher and growth of the fish significantly less in the diet containing the soybean oil meal.
3. Vitamin supplementation appears to have no significant growth benefits when fed in amounts exceeding the single level of supplementation.
4. Fish receiving meat supplements deposited greater levels of protein than did fish fed the all-meal diet. This growth increase was most apparent during the first four weeks of feeding. After this time the biweekly percent gains of the fish from the all-meal diets were as good, if not better.
5. A sparing action was produced on the protein requirements of the fish by increasing the caloric level of their diet by the addition of peanut oil. Diets fed at 2,350 calories per kilogram of food and at a protein calorie-to-energy calorie ratio of 1:1.35 appear to be optimum under the conditions of this experiment.
6. Histopathological examination of fish fed the all-meal diet and supplemented with peanut oil, a soft fat, revealed no fat deposits in

the livers. In these fish extra dietary fat was reflected in increased visceral fat only. In contrast fish fed the control meat diet exhibited definite fat deposits within the livers and little if any visceral fat. The deposition of visceral fat probably represents a more desirable storage depot physiologically than does the liver. Ceroid deposits were also present in the livers of the meat fed fish, which was probably due to the feeding of highly rancid raw salmon viscera.

The final report on the 1963 diet trials scheduled for completion in March 1964, will conclude this work unit.

## ENVIRONMENTAL FACTORS

### Oxygen demand

The oxygen requirements of 5-inch, 6-inch, and 7-inch chinook salmon fingerlings of the same age were determined in 1962. The larger fish required about 25 percent more oxygen per pound of fish per gallon of water flow than the smaller fish. The difference in oxygen requirement within the same age group is probably due to the different metabolic rates of the fish.

In 1963 the oxygen requirement of chinook salmon during normal growth was determined. The older and larger fish required less oxygen per pound of fish than the younger and smaller fish. This effect is apparently linear, but there is an abrupt change in oxygen requirement as the fish approach 5 grams in weight (89 fish per pound) when the oxygen requirement per pound of fish levels off.

At 53° F. inactive chinook salmon fingerlings weighing 1.23 grams required 0.61 parts per million oxygen per pound of fish per gallon of water flow. Up to 5 grams in weight the oxygen requirements were found to decrease .051 ppm for each gram increase in weight. Beyond 5 grams in weight and up to the last measured values for 14-gram fish the oxygen requirements were found to decrease .0082 ppm for each gram increase in weight.

In 1964 the oxygen requirements of chinook fingerlings will be investigated at different

temperatures. The effect of diet on oxygen requirements will also be explored. It is conceivable that the measurement of oxygen requirement of fish may be useful for assessing hatchery success. Human metabolic rate has been determined by measuring the oxygen consumption of resting subjects. The measurement should prove to be easier with fish.

### Water reclamation

Preliminary experiments were conducted to determine some of the problems associated with rearing fish in continuously re-used water. A recirculating system was set up which contained 117 gallons of water circulated at the rate of 9 gpm by a motor-driven pump. Continuous aeration of the water assured an oxygen level of near saturation at all times. Installation of a heat exchanger cooled by 53° F. water was necessary to hold the temperature in the system within tolerable limits. Experiments were conducted with chinook fingerlings in well water at pH 8.0 and in creek water at pH 7.3.

In a test utilizing well water, fish became distressed after 28 hours in the recirculating system. The ammonia level in the water at that time was 1.03 ppm as determined by direct nesslerization.

Chinook fingerlings appeared normal and fed actively for over two weeks in recirculating creek water even though the ammonia level increased rapidly. On the 17th day of the experiment the ammonia level was 27 ppm and the pH had risen to 8.05. At that time the fish showed signs of distress and ceased feeding although histological examination of a sample of fish revealed no gross abnormalities.

After a maximum of 27.6 ppm ammonia was attained on the 19th day of the experiment, a condition developed in the re-use system which caused a decline in both ammonia level and pH. When the experiment was terminated on the 24th day due to the moribund condition of many of the fish, the ammonia had decreased to 14.6 ppm and the pH to 6.7. The cause of the decline is not known but a bacterial growth is suspected. An attempt to reproduce the condition in the system with no fish but with ammonia in an amount comparable to that produced by fish failed.



The results of the test utilizing well water were as anticipated, but the results of the creek water experiment appeared unreasonable. Experiments will be initiated early in 1964 in an attempt to substantiate the results of the 1963 tests.

## DIFFERENCES IN FINGERLING SALMON

### Physiological and chemical differences

This program includes the characterization of hatchery fish by correlating measured characteristics of fingerling fish with the returns of the adult. Characteristics which do not show differences will be discarded and those which reflect differences will be measured each year during a four-year hatchery evaluation of the lower Columbia River hatcheries. The objective is to measure quality of hatchery fish.

The 1962 investigation revealed that there are measurable differences in body components, blood components, and physical condition. The measurements which showed differences were continued in 1963. New measurements were added to determine whether other measurable differences could be detected. In 1963, the measurements used to determine differences between fish were weight, physical performance, gross pathological examination, hematocrit levels of the blood, number and size distribution of the blood corpuscles, blood plasma level of protein, glucose, cholesterol, calcium and phosphorus and body composition including water, protein, lipids, ash and glycogen.

In 1963, 24 lots of chinook fingerlings from 16 hatcheries were analyzed to determine whether there were discernible differences among them. Three categories of fish were encountered. It appears that the difference found were due to cultural practices and/or environment. Each category showed a characteristic level of blood cholesterol and body fat. The fish samples from one of these groups had a lower percent of small blood corpuscles than the other two categories.

The results obtained in the measurement of differences show that an evaluation of such

differences between hatcheries is dependent on definition of what body interrelations are necessary for a balanced state. When the percentage composition of one component varies, then the level of other components vary, also, to compensate for the change. The analysis of such data probably cannot be obtained by simple statistical comparisons of the interrelations existing between the various components because the body balance is usually not that simple.

A direct correlation was found between body lipids and plasma cholesterol of the chinook fingerlings examined in the hatchery evaluation study. A direct correlation was found between fish weight and physical performance and between fish weight and body protein. There was no correlation between fish weight and body fat. These analyses indicate that these small fingerlings were in an extremely active metabolic state with little fat being deposited, therefore, either protein deposited or total weight would be a measure of fish condition for these fish. Protein deposition would be a better measure since weight alone would not reflect body catabolism or an emaciated condition.

Coulter Counter cell size frequency distribution plots of the corpuscles in blood samples from fall chinook fingerlings have revealed considerable variation among hatchery populations. The zone of variation is confined primarily to the first twenty-five percent of the cell size range. This size range lies between 100 cubic microns and 200 cubic microns, and has been designated the small cell portion of the range. The percentage of the total cells found within the small cell range has been found to correlate closely with the performance rating for the same hatchery populations. The relation between the percentage small cells and the performance rating is an inverse one in which the performance increases as the percentage of small cells decreases. We have assumed that the performance rating is a good index of quality. We assume, also, from the inverse correlation demonstrated between the performance rating and the percentage of small cells that the percentage of small cells in the blood may be a measure of fish quality. Our data, at this time, indicate that fish having a percentage of small cells ranging from 10 to 30 percent of the total may be expected

to have a higher stamina than those with a percentage of small cells ranging from 30 to 50 percent.

Two sets of data have been compiled on blood corpuscle size distribution patterns. One set was derived from fish prior to stressing in the stamina tunnel, the other from fish following stressing in the stamina tunnel. In comparing the two sets of data, it has been observed that there was usually a larger percentage of small cells in the post-stress sample of fish than there was in the pre-stress sample from the same population.

This difference indicates that an increase in the percentage of small cells is the expected response to stressing. It was further found through experimentation that the time of increase in the percentage of small cells is within the first hour following the commencement of stress and that the response is measurable and stable for at least twenty-four hours after stressing. The manufacture of new cells of this magnitude in such a brief span of time is unlikely, thus we assume that they are merely released from storage where they have been held in reserve to meet the demands of a stress condition.

Efforts to relate physical performance with measurements other than protein, weight, or corpuscular size were unsuccessful. Final evaluation of the significance of the differences between the chinook fingerling will be undertaken when the adults return.

The electrophoretic separation of protein appears to be one of the best procedures for determining differences between fish. The measurement of protein fractions will be emphasized in 1964 since all of our preliminary studies to date indicate that the proteins are preferred indicators for showing differences in the blood.

Plans for 1964 are to repeat the measurement of 1963 with the exception of calcium and phosphorus determinations on blood plasma. Greater emphasis will be given to electrophoretic separation of plasma proteins and to a more detailed pathological examination of the internal organs. Exploratory tests are being conducted to determine whether transketolase

levels of blood plasma should be included in measuring differences. Muscle protein composition, hemoglobin composition and oxygen demand are also being considered as criteria of differences.

#### Stamina tunnel

A manuscript is nearing completion giving a description of the stamina tunnel, the procedure of operation, the methods of evaluation, and the controllable variables involved in testing.

Stamina tests were conducted on fish samples from the 1963 hatchery evaluation program. Definite differences were found between the performance of samples from the different hatcheries.

Fish samples for the 1962 hatchery evaluation program had varied widely in the number of days since feeding. A study on the effect of starvation on swimming ability was conducted to determine the validity of results from stamina tests on those fish. There was no decrease in stamina until between the fifth and seventh day without food for fish averaging one gram. Fish averaging three grams performed well for at least four days without food. The sizes of these fish were slightly smaller and slightly larger than the hatchery evaluation samples. The handling procedure of the samples at the individual hatcheries was standardized to eliminate this potential variable.

A study on the effect of re-testing fish which had previously been run through the stamina tunnel revealed that at least three days of rest after the initial run was required before the original performance level could be regained.

During 1962, a normal curve was determined for the effect of fish size on performance using samples from a single conventional raceway. Biweekly stamina tests were conducted during the spring of 1963 on fish populations from four different pond environments; the circular, conventional raceway, recirculating raceway, and rectangular-recirculating pond type. All the fish were being fed the same diet and were in the same condition of health. The studies were designed to test the reliability of the normal

curve and to study the effect of the rearing environment on small fingerlings. No major differences were found in the performance of any of the groups. It appears that the type of rearing environment is of minor importance to stamina in small fingerlings. Differences in performance due to nutrition and disease are detectable in small fingerlings.

Biweekly tests were conducted during the summer on fish samples from the rectangular-recirculating ponds being reared at two different caloric levels. No significant difference in the performance index was found between the two groups of fish during this time. It had been expected that the larger size of the high-calorie fish and the increased energy calories available would produce significantly higher performance results above that of the low-calorie group before the rearing season was over. The flow patterns of the rearing pond types used for this experiment do not restrict performance as the fish size increases. Both groups, and especially the high-calorie group, went through a period of extremely rapid growth. This rapid growth soon produced an overcrowding effect in the rearing environment accompanied by a buildup in metabolic waste products. This overcrowding effect probably was the cause of the reduced swimming ability of these fish.

Stamina tests were conducted on samples of the 1963 experimental diet trials at their completion. Performance indices were high in all diets and no significant differences were found between diets. Results varied widely within some diets, possibly due to an environmental effect from raising large fingerlings in small, six-foot circular tanks.

A performance rating has been developed which will measure the energy expended by the fish during a stamina test. The standard hydraulic formula for the drag of an object in a fluid has been adapted to evaluate the swimming ability of a group of fish in the tunnel.

There are several advantages in the use of the performance rating over the performance index. The actual energy used by the fish gives a more accurate evaluation of its stamina than does the time criteria employed for performance

index. The consideration of viscosity allows the comparison of tests conducted at different water temperatures. Fish which have no significant increase in swimming ability with an increase in size would have the same performance index while the performance rating would actually decrease.

Comparisons of the two methods of evaluation are shown in figure 1 and figure 2 prepared from data on the 1962 pond environmental studies. Above an average weight per fish of approximately 5 grams, an increase in swimming ability with increased size will produce a higher performance index and the performance rating will remain about the same. Below 5 grams per fish, there appears to be little difference in the two methods of evaluation. Variability existing in the stamina of different groups of small fingerlings is masked to some extent by a rapid acceleration in swimming ability as the fish grow. A plus or minus deviation from a standard curve determined for the performance index may well be the best method of evaluation for these fish. A major advantage of the performance index is the speed with which it can be calculated.

#### FINGERLING CHARACTERISTICS AFFECTING ADULT SURVIVAL

##### Effect of two caloric levels in diet on quality of fingerlings produced

The initial phase of an experiment to determine the effect of two levels of caloric intake by fingerlings on adult survival was completed this year. Two groups of 170,000 each of marked fall chinook salmon were released in Abernathy Creek in August of 1963. Both groups were fed comparable diets with the exception that in the high calorie diet 7 percent of peanut oil was substituted for 7 percent of water in the meat-meal mixture. The addition of the peanut oil resulted in better gains, more efficient protein utilization, greater disease resistance, and similar hematology. Pathological examination disclosed a high concentration of fat in the liver of high-calorie-fed fish. Such fat concentrations are typical for most hatchery fish. In terms of hatchery performance the high calorie diet produced a fish of superior quality. Return of the adults in 1966

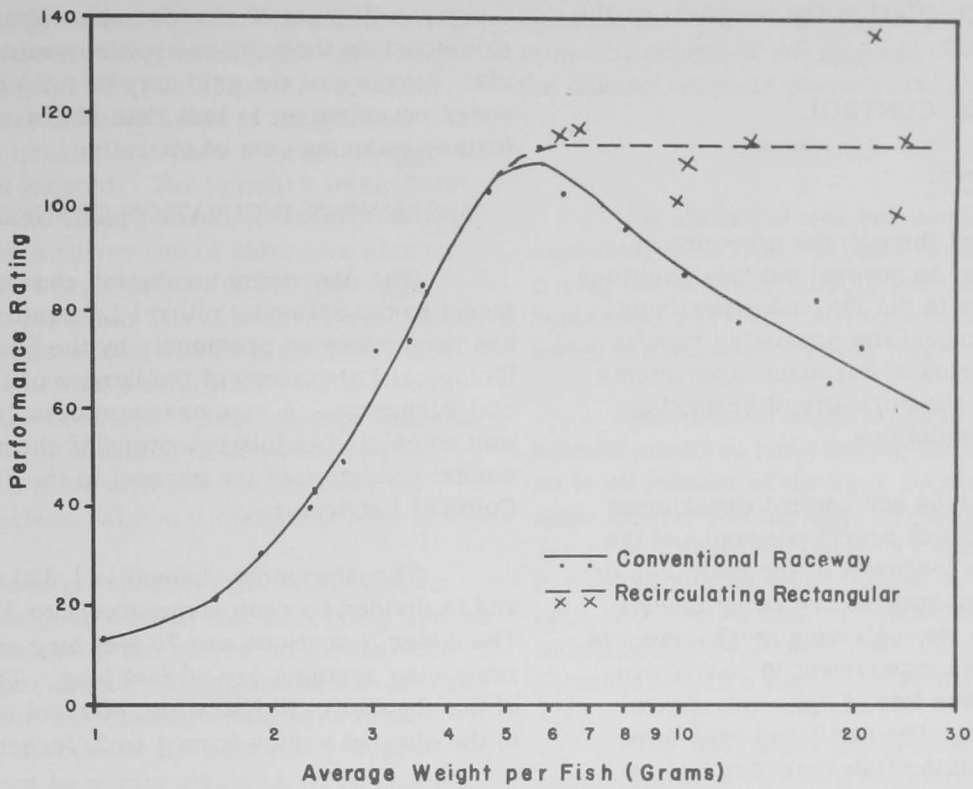


Figure 1:--Normal curve for performance index showing depressing environmental effect of raceway ponds after fish reach 5 grams in weight.

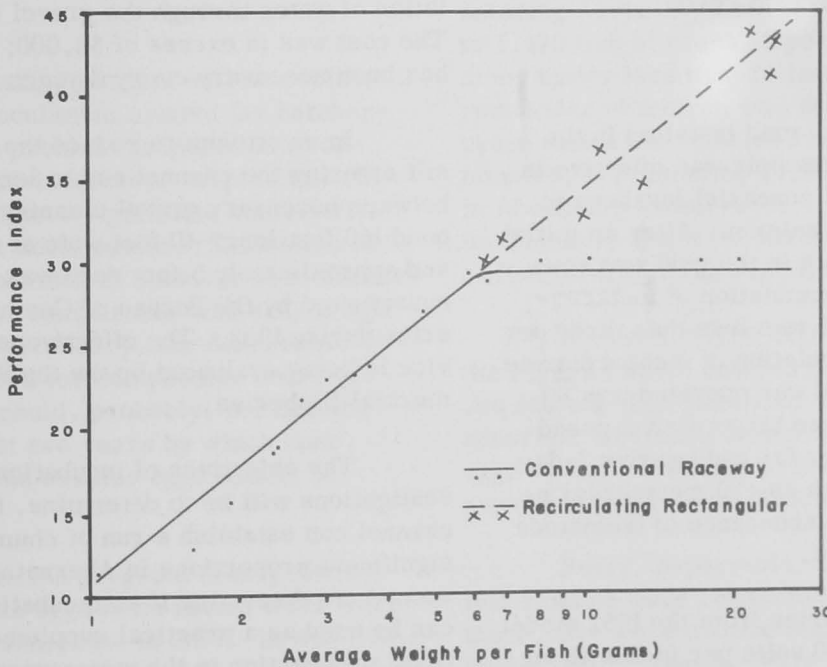


Figure 2:--Plot of performance rating showing same data as figure 1. Note inferior performance from raceway pond.



will determine the effect of the two diets on the survival of the fish.

## SEX CONTROL

### Method development

Sex control through the exposure of green salmon eggs to several estrone solutions proved ineffective in the 1962-63 experiment. The inability to repeat the promising results of the 1961-62 series of hormone experiments was attributed to the difficulty of preparing standard estrone solutions.

In the 1963-64 sex control experiment the pH of the media of sperm passage and the differential in the longevity of the male and female gene-bearing sperms are being investigated as possible sex-selecting mechanisms in fertilization. This experiment is now in progress. The various lots of eggs and sperm have been treated. The fertilized eggs have been incubated and the fish reared to sexing size. Sexing is in progress but data are not yet available as to the success of the experiment.

## CERCARIAL CONTROL

### Prototype tests

A new electrical grid installed in the Abernathy Creek water supply was effective in reducing the number of cercarial invaders in pond-reared fingerling salmon. After an initial erratic hydraulic pattern in the grid was controlled by baffling, accumulation of metacercariae in pond-held fish was less than three per week. The total accumulation of metacercariae during the time the grid was operated was 80 per fish. Cercariae-free target fish exposed to untreated creek water for consecutive 7-day periods acquired as high as 170 metacercariae in a week affirming the abundance of trematode cercariae in the stream.

The new grid varies from the 1962 model in that it operates at 310 volts per inch with an exposure period of 0.5 seconds and has an adjustable capacity of from 2 to 12 cubic feet per second of water flow. Due to improved design

the grid will treat 12 cfs. for approximately the same cost as the previous model would treat 5 cfs. Portions of the grid may be turned off if water requirement is less than 12 cfs., thereby further reducing cost of operation.

## ABERNATHY INCUBATION CHANNEL

The Abernathy incubation channel is adjacent to the Salmon-Cultural Laboratory and has been operated previously by the Seattle Biological Laboratory of the Bureau of Commercial Fisheries. A memorandum of understanding was completed in July transferring the experimental operation of the channel to the Salmon-Cultural Laboratory.

The Abernathy channel is 1,820 feet long and is divided by drop structures into 32 sections. The upper 11 sections are 70 feet long and the remaining sections are 50 feet long. The bottom of the channel is 10 feet wide. Gravel size used in the channel varies from 1 to 2 inches.

An excessive silt load had accumulated in the channel and it was necessary to remove the gravel from the channel, wash, screen, and return it in order to insure an adequate circulation of water through the gravel for the eggs. The cost was in excess of \$2,000; This cleaning has been necessary every 2 years.

In an attempt to reduce the amount of silt entering the channel and to lengthen the time between necessary gravel cleanings, a settling pond 160 feet long, 40 feet wide at the bottom, and approximately 5 feet deep was designed and constructed by the Bureau of Commercial Fisheries during 1963. The effectiveness of this device is being evaluated by the the Bureau of Commercial Fisheries.

The objectives of incubation channel investigations will be to determine, first, if the channel can establish a run of chum salmon of significant proportions in Abernathy Creek and, second, to determine if an incubation channel can be used as a practical supplement to artificial propagation in the maintenance of hatchery-supported fall chinook salmon runs.



Two species of salmon are suitable for experimentation in the channel, the chum and the fall chinook. The problem with the chum salmon is that the native run is insignificant and another consistent single source of eggs has not been located. The primary immediate objective in the chum salmon investigations will be to develop a native run of sufficient size to support paired experiments of 500,000 eggs each. To accomplish this objective, eyed chum eggs from other areas will be planted in the channel for at least 2 additional years. Once a native run has been established an experimental program will be initiated to determine procedures for the most efficient operation of the channel for chum salmon propagation.

The second species, the fall chinook, also presents problems in that it is being reared at the station and is the subject of extensive marking experiments throughout the area. Fish released from the channel would have to be marked and marks for the next two years, at least, will not be easily obtained. The channel should accommodate the two species without too many complications as both the spawning times and migration times are different. Space for segregation and incubation in the channel is not a problem.

The incubation channel will be evaluated as an auxiliary incubation system for hatchery operations. The problem proposed is to determine if channel-incubated, surplus fall chinook eggs will make a significant contribution to the subsequent adult return. Evaluation is to be made by comparison of survival with hatchery-propagated fish of comparable size and/or age to determine, if necessary, the differential development required for comparable survival. Such a program could, probably, not become active for at least two years by which time, both the marks and surplus eggs should be available.

To initiate the program nearly 160,000 eyed chum salmon eggs from native stock were planted in the channel late in 1963. In addition, approximately 1,000,000 eyed-eggs will be supplied from the Quilcene National Fish Hatchery and planted in the channel in February 1964.

Channel survival from these eggs will be determined by counts of the migrants as they leave the channel on their downstream migration.

## GENERAL

No abnormal weather conditions were encountered in 1963. Stream flows held up well during the summer months and the rainfall in September was sufficient to provide adequate water for the adult chinook migration.

Mortalities in the stock of fall chinook salmon received from Spring Creek NFH amounted to 40 percent of the eggs received. The losses were incurred in the eggs as premature hatch due to soft shell and in the fry and fingerlings due to coagulated yolk. Of the 3,000,000 eyed eggs received, a total of 1,806,000 fingerlings weighing 33,500 pounds were liberated in three releases, April, May, and August, 1963.

The first adult run of chinook salmon of significant size returned to Abernathy Creek this fall. Complete diversion of the upstream migration at the holding pond and semi-weekly patrol of the three miles below the hatchery has enabled a complete accounting for the run entering Abernathy Creek. The run amounted to 1,470 fish of which 50 percent were precocious males less than 28 inches in length. The remainder of the run was equally divided between males and females, but 46 percent of the females, representing 1,000,000 eggs, spawned in Abernathy Creek in the 1-1/2 miles of stream immediately below the hatchery. This number of fish saturated the available spawning area.

A total of 1,100,000 eggs was taken from the females which entered the holding pond. Losses amounted to 3 females or 1.2 percent. No abnormal mortalities were encountered in the eggs or fry. The loss to feeding fingerlings amounted to less than 10 percent.

Forty, 3-year-old, marked fish from the 1961 release were recovered from the run. Of these, five were mature females averaging 30.5 inches and 17 pounds. Five marked, 2-year-old jacks, also, were included in the run.

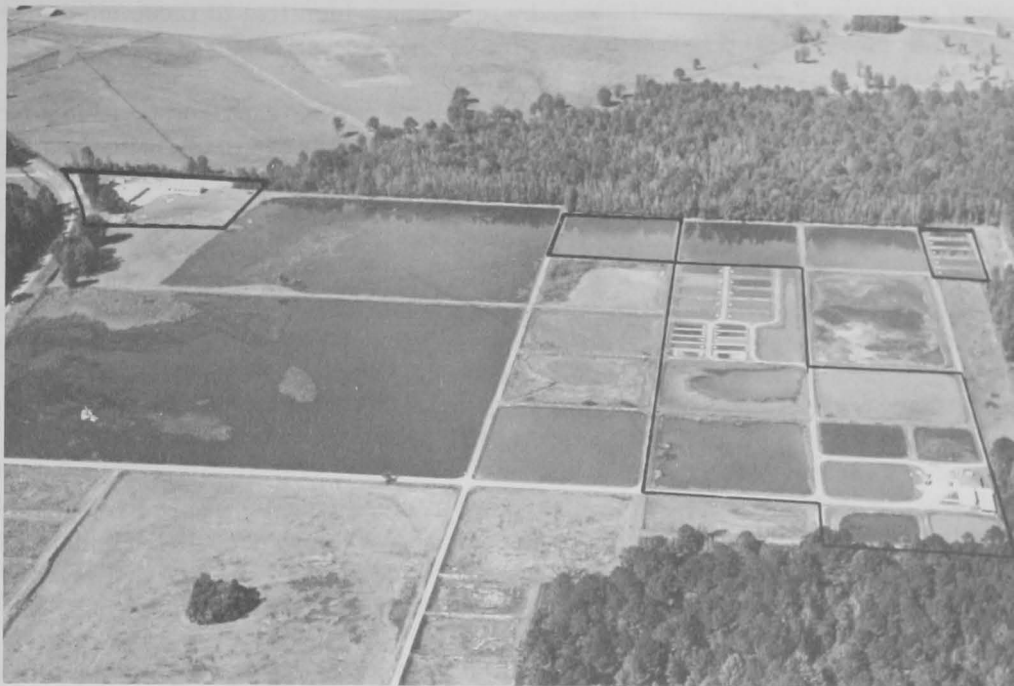
## STAFF

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Mr. Harry H. Chenoweth, Hydraulic Engineer  
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Mr. Joseph W. Elliot, Chemist  
Mr. Bobby D. Combs, Fishery Biologist  
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# SOUTHEASTERN FISH CULTURAL LABORATORY

Marion, Alabama

Kermit E. Sneed, Chief



The area of the Marion National Fish Hatchery occupied by research. The areas being used at present are enclosed in the dark lines.

## HIGHLIGHTS

The first reproduction of blue catfish at this laboratory was obtained.

Flathead catfish reared from fry in hatchery brood ponds were spawned with hormones. These fish, two and three years of age and weighing 0.7 to 1.2 pounds each, are the youngest and smallest flathead catfish that, to our knowledge, have been sexually mature and have been spawned.

Gonadotropic hormone-cholesterol pellets influenced the gonadal development of sexually immature goldfish.

The synthetic steroids Enovid and Norlutin inhibited the ovarian weight of goldfish, but Delalutin and Provera increased ovarian weight when administered orally to sexually immature goldfish. Delalutin increased gonadal

weight in male goldfish, but the other three steroids had no apparent effect.

Best growth was obtained with "purified" diets that contained 10 percent alpha cellulose flour (dietary bulk) when fed to channel catfish fingerlings.

The "binding" of casein hydrolysate and tryptophan with beef tallow, hydrogenated corn oil, hydrogenated vegetable oil, lard or corn oil in amino acid test diets for channel catfish was tested. At the end of a 21-day feeding trial, no conclusive growth was obtained.

Effect of diet, environment and disease on the electrophoretic pattern of the sperm proteins of channel catfish was studied. These environmental factors appear to change the ratio of the serum proteins.

No correlation was found between blood glucose levels of pyridoxine-deficient fish and the tetany-hyperirritability symptoms.

Chromosome numbers of channel catfish appear to be in the 52 to 56 range.

Six hybrids of five species of catfish were obtained by hand-fertilization methods. The methods developed may allow us to obtain additional hybrids next year for comparative performance studies.

#### EFFECT OF HORMONES ON SPAWNING OF FISHES

Because of limited facilities, no work units were started or completed on this project in 1963. Hormones were employed to obtain eggs and fry for hybridization studies and nutrition experiments described elsewhere in this report. The hormones were injected as follows:

<u>Hormone</u>	<u>Dosage</u>	<u>Frequency of Injection</u>
Human chorionic gonadotropin	500-600 I.U./pound	48-60 hours
Fish pituitary	2 mg./pound	48-60 hours

Injections were continued until the fish spawned or the eggs were ruined. Most fish spawned after the first or second injection but in a few instances four injections were required.

##### Channel catfish

Hormone-induced spawning of hatchery-reared channel catfish was more predictable at this laboratory this year than in previous years, but still not as successful as at some hatcheries. Research will be continued in 1964 to determine the cause of the difficult and unpredictable spawning observed at this laboratory.

##### Blue catfish

The first reproduction of blue catfish at this laboratory was obtained last spring. Sexually mature blue catfish were obtained from local rivers and spawned with hormone-induced

spawning techniques identical to those employed with channel catfish and flathead catfish.

The eggs were hatched and the fry reared by techniques identical to those used in the culture of channel catfish.

Blue catfish captured from local rivers were much less hardy than channel catfish and flathead catfish captured under similar conditions. Most of the blue catfish captured and spawned this spring died in the spawning facilities or hatchery ponds. Mortality of channel catfish and flathead catfish after identical treatment was very low. The blue catfish fingerlings reared in the hatchery handled well.

##### Flathead catfish

Flathead catfish reared from fry in hatchery ponds were spawned with hormones last season. These fish, two and three years in age and weighing 0.7 to 1.2 pounds each, are the

youngest and smallest flathead catfish that, to our knowledge, are sexually mature and have been spawned.

#### EFFECT OF HORMONES ON PRECOCIOUS OR RETARDED DEVELOPMENT

One work unit was started and completed in 1963. The results of this research is summarized below.

##### Hormone-cholesterol pellets

A hormone-pellet experiment conducted in 1960 was repeated on sexually immature goldfish, the object being to speed up the sexual maturity of the female. Six treatments involved human chorionic gonadotropin, pregnant mare serum, three pituitary fractions (P<sub>1</sub>, P<sub>2</sub>, and P<sub>3</sub>; acid extracts precipitated with acetone), and the pituitary residue, each bound with cholesterol

and pressed into pellets. Cholesterol-only pellets and no pellets served as controls. The pellets were inserted into the body cavity of the sexually immature female fish on November 1, 1962.

The fish were stocked into aquaria and fed dry feed at the rate of 2 to 3 percent body weight per day six days a week. The water temperature in the aquaria ranged from 69° F. to 57° F. during the experiment. The experiment was concluded on February 18, 1963.

The analysis of the variation in mean gonosomatic index indicated that the difference between some groups was highly significant ( $P=0.01$ ). Differences in the mean gonosomatic index between the cholesterol-only pellet injected fish and the HCG,  $P_1$ ,  $P_2$ , and PMS treated groups were not significant. When compared to the same control, the  $P_3$  treatment significantly ( $P=0.01$ ) inhibited ovarian development as did the pituitary residue treatment ( $P=0.05$ ). When each hormone-treated group was compared to the control group which received no pellet, the HCG-cholesterol pellet, the  $P_1$ -cholesterol pellet, and the PMS-cholesterol pellet groups had the highly significant ( $P=0.01$ ) effect of accelerating ovarian development.

When the three most effective hormone-pellet preparations (HCG,  $P_1$ , and PMS) were compared among themselves no significant differences were found between their effects.

The observation that the cholesterol-only injected group had a higher mean gonosomatic index than the group receiving no pellet (this difference was not tested for significance) may suggest that additional tissue-growth-promoting steroids were biosynthesized by the fish via the injected cholesterol, but the most likely explanation is that the growth was due to the stress of the injection and irritation in the body cavity with the fish producing endogenous gonadotropins in greater amounts.

#### SEX REVERSAL AND STERILITY OF FISH

A work unit (FR-1603:1) entitled "Effect of four orally administered synthetic steroids on the development of gonadal tissue of goldfish

(*Carassius auratus*)" was completed and the results are in manuscript. This research problem was conducted by James C. Anderson, student at the Marion In-service Training School, and directed by laboratory personnel.

Anderson found that Enovid had highly significant ( $P=0.01$ ) inhibitory effect on ovarian weight in goldfish, and that Norlutin produced slightly less inhibitory effects. Delalutin and Provera caused significant increases in ovarian weight, the opposite of what had been expected. Delalutin also had the highly significant effect ( $P=0.01$ ) of increasing testicular weight in goldfish. No mortality occurred among the 150 fish in the experiment and no harmful effects were noted from feeding the steroid preparations.

#### THE NUTRITION OF THE CATFISHES

Three work units on the nutrition of the channel catfish were started and two were completed in 1963. The results of this research are summarized as follows:

Alpha-cellulose flour---Two series of purified diets containing 0, 10, 20, 40 and 50 percent dietary bulk were prepared. In one series, equal diet volumes were maintained by substituting an equal weight of bulk for water and in the other series an equal percent of solids was maintained by adding equal weights of water and bulk. The other nutrients (protein, carbohydrate, lipid, vitamins and minerals) were available to the fish in equivalent amounts in all 10 diets.

In both series, the best growth was obtained with diets that contained 10 percent bulk. Diets with no bulk or 20, 40, or 50 percent bulk resulted in retarded growth. Little difference in growth was observed between diets with constant volume and constant solids.

The results of this study of dietary bulk will be incorporated into a paper entitled "Purified diets for channel catfish nutritional research" (in manuscript).

Fats in amino acid test diets---Research was continued to devise a suitable amino acid test diet for channel catfish. Diets that have given



growth in trout and salmon nutritional studies have not given growth when fed to channel catfish.

Our opinion is that the nutrients in the crystalline amino acid test diets pass through the stomach and small intestine so rapidly that little is absorbed. Previous experiments with high levels of binders (CMC or agar), solids or bulk did not increase the retention time of the food in the stomach or intestine.

Fats have been reported to reduce peristaltic movements of the digestive tract and increase time required for digestion. In this test series we bound the amino acids (casein hydrolysate and tryptophan) in fat (beef tallow, hydrogenated corn oil, hydrogenated vegetable oil, lard and corn oil) and then added a salt mixture, white dextrin, complete vitamin mixture and alpha cellulose flour. These diets were bound with a water-CMC mixture. A control diet was devised that contained whole casein and corn oil instead of the casein hydrolysate and tryptophan.

At the end of 21 days, only a "suggestion" of growth was observed in those groups fed the amino acid test diets but approximately 50 percent increase in weight occurred in the group fed the casein-test diet.

Pyridoxine requirement of channel catfish--Research was conducted to determine the minimum requirement for pyridoxine in the diets of rapidly growing channel catfish. A series of 10 diets was devised that contained "vitamin-free" casein, corn oil, white dextrin, salt mixture, alpha-cellulose flour, pyridoxine-free vitamin mixture and bound with an agar-water mixture. The level of pyridoxine HCl in this series of 10 diets ranged from 0.0 to 3.5 milligrams per 100 grams of feed (dry weight).

An undiagnosed "disease" interrupted this experiment before the dietary pyridoxine HCl requirement could be determined. This experiment will be resumed in 1964.

#### CHARACTERISTICS OF BLOOD OF FISHES

Three work units were completed in 1963. The results are summarized as follows:

Effect of environment and disease on the electrophoretic pattern of the serum proteins of channel catfish--Preliminary research on the effect of diet, environment and disease on the serum proteins of channel catfish was completed. This research was conducted by Arden J. Trandahl, student at the Marion In-service Training School, and directed by laboratory personnel.

Serum protein samples were collected from 14 groups of channel catfish from different environments and various physiological conditions. The blood serum was analyzed in a Beckman Model R Electrophoresis System.

Trandahl found that the albumin/globulin ratio of channel catfish in good physiological condition averaged approximately 0.9. The A/G ratio of fish that were "diseased" or that had been fed a protein-free diet averaged less than 0.8. The A/G ratio of fish that had been fed "purified" diets in aquaria was greater than 1.0.

Although the results of this research were not conclusive because of an insufficient number of samples, it indicated that the A/G ratio, albumin-globulin electrophoretic pattern, and the quantity of albumin and globulin may be developed in conjunction with other determinations as a tool to distinguish good diets from inferior diets, good environmental conditions from poor environmental conditions, and healthy fish from diseased fish.

Effect of pyridoxine deficient diets on blood calcium and glucose of channel catfish--Research was conducted to determine if the level of glucose and calcium in the blood of pyridoxine-deficient fish is involved in the hyperirritability-muscle tetany symptom. We observed that the blood glucose level of channel catfish 16 hours after being fed either a pyridoxine-deficient diet or a vitamin-complete diet was approximately 40 milligrams percent. However, within one hour after food was offered, the blood glucose level of the vitamin-complete group was elevated to 90 milligrams per 100 milliliter of serum, but the blood glucose level of the pyridoxine-deficient group remained near 40 milligrams percent. This difference could have been due to the fact that the fish fed the vitamin-complete

diet readily ate this food, but that the fish fed the pyridoxine-deficient diet ate little or not at all. However, in previous experiments the pyridoxine-deficient fish ate well throughout the experiment and still developed the deficiency symptoms.

We found 16 hours after being offered feed that the blood calcium level of the pyridoxine-deficient groups averaged 10 to 11 milligrams percent, and the blood calcium level of the vitamin-complete groups averaged 15 to 16 milligrams per 100 milliliters of plasma. Whether or not blood calcium is involved in the hyperirritability-muscle tetany symptom of pyridoxine-deficient fish will require additional study, but it seems reasonable to assume that this much change in the blood calcium would certainly change the permeability of the cell membranes, thus accounting for the tetany symptoms.

#### Aminograms of channel catfish blood---

In cooperation with the Western Nutrition Laboratory, the free amino acids in the blood stream of channel catfish were determined. The basic assumption is that the free amino acids in the blood stream when anabolic processes are exactly balanced by catabolic processes in a reflection of the amino acid requirements of that species.

Serum samples were prepared from the blood of fish that had been starved 24, 48, 72, 96, and 130 hours. After the amino acid patterns had been analyzed, it was not possible to plot the trough for each amino acid when anabolic processes are balanced by catabolic processes. Recent work in pigs indicates that this trough may appear three to six hours after food is ingested. With these short-gutted fish, the trough may have been eliminated before the first sample of blood was removed. Research on the amino acid requirement of channel catfish will be continued in 1964.

#### STUDIES ON THE DESIRABILITIES OF CATFISH HYBRIDS

Attempts were made to artificially produce nine different types (including reciprocal

matings) of catfish hybrids from the following species: channel catfish (Ictalurus punctatus), blue catfish (Ictalurus furcatus), flathead catfish (Pylodictis olivaris) and white catfish (Ictalurus catus). Acceptable survival has resulted from six of these crosses. Little success resulted when the flathead catfish female was in the cross. Hybrid vigor may be present in most of the crosses, but at this time appears greatest in the white catfish male X the blue catfish female. Also, this hybrid has a body form (short, stocky, full-fleshed) that makes it appear promising as a hybrid valuable as a commercial fish. Unfortunately, facilities did not permit adequate comparison of growth, survival and hybrid vigor this year.

Hormone spawning, fertilization techniques and incubation methods (to be described at a later date) appear adequate for the production of large numbers of hybrids of any of the catfishes that do produce a desirable cross.

Present plans call for including the bullheads in next year's experiments with hybrid catfish. Also, we plan to keep the present hybrids to determine which ones are fertile to attempt some back-crossing.

A few hybrids and the mother species were examined with Dr. George Moore, Oklahoma State University and a preliminary key was prepared.

#### SIDELIGHTS

Comparison of growth rate of male and female channel catfish---Research entitled "Comparison of growth rate of male and female channel catfish, Ictalurus punctatus" was completed in June. This problem was conducted by John A. Beaver, student trainee at the Marion In-service Training School, and directed by our staff.

In both "hatchery" ponds and "wild" environments, the growth rate of male channel catfish exceeded that of female channel catfish from fingerling through sexual maturity. The data do not reveal whether or not the male is more aggressive, a better forager, or a better converter of food.

A preliminary key to four species of catfish and to the young (2-4 inches) of five of their hybrids

Kind of Fish	Anal Rays	Gill Membranes	Caudal Fins	Gape : Snout	Std. Length : Head Length
Flathead Catfish ( <u>Pylodictis olivaris</u> )	14-16	Broad overlap	Slightly notched	Half	3.1
Channel Catfish ( <u>Ictalurus punctatus</u> )	25-27 (tips rounded)	Slight overlap	Lobes pointed in young; fork intermediate between BC and WC	Greater	3.7
Blue Catfish ( <u>Ictalurus furcatus</u> )	32-34 (tips straight)	Moderate overlap	Deeply forked	Equal	3.6
White Catfish ( <u>Ictalurus catus</u> )	20-22	No overlap	Fork moderate; tips rounded	Less	3.4
Flathead Catfish Male X Channel Catfish Female	19-20	Strong overlap	Moderate fork; rounded lobes	Equal or less	3.0
Flathead Catfish Male X Blue Catfish Female	21-24	Strong overlap	Forked as WC; lobes rounded	Less	3.1
Blue Catfish Male X Channel Catfish Female	28-30 (tips straight)	Slight overlap	Fork intermediate	Equal	3.5
White Catfish Male X Channel Catfish Female	23-24	Slight or no overlap	Fork intermediate; lobes slightly rounded	Equal	3.4
White Catfish Male X Blue Catfish Female	25	Slight or no overlap	Forked as CC; lobes more rounded than BC X CC	Less	3.6

From the data that has been collected in the three groups of channel catfish, information has been obtained that may be helpful to federal and State hatcheries as well as commercial hatcheries. When a hatchery is planning to stock fingerlings for broodstock, grading the fish according to size and stocking only the large fish, which is a common practice on most hatcheries, might result in an excess of males. By grading the fingerlings according to size, it is possible for the hatchery to rear broodstock with a larger percentage of males or females, whichever is needed. The data are too limited to indicate certainly the natural sex ratio in channel catfish populations, but these data do strongly suggest that the largest fish in a normal hatchery population are males. Thus, selective grading might possibly be employed to obtain groups with disproportionate sex ratios.

"Bacterial" growth in channel catfish and flathead catfish egg masses---A "bacterial" growth in channel catfish and flathead catfish egg masses in a mechanical hatching trough was observed last season. This "bacterial" growth destroyed the adhesive material joining the eggs thereby causing disintegration of the egg mass. The "bacteria" did not kill the eggs directly but instead permitted piling-up and subsequent suffocation of the eggs on the bottom of the trough.

The condition was characterized by the water becoming cloudy and foamy followed by disintegration of the egg masses after approximately 24 hours.

The condition was corrected by thoroughly cleaning and flushing the trough with approximately 5 ppm acriflavin. Since eggs, fry and fingerling catfish are very tolerant to acriflavin, this treatment can be used periodically without danger.

Chromosome numbers in channel catfish---During the past catfish spawning season, em-

bryo squashes were made in an effort to perfect a technique for determining chromosome numbers in catfish. Carnoy's fixative at the 8 to 64-cell stage followed by aceto-orcein staining showed the greatest promise. Weak solutions of colchicine did not prove advantageous at this stage of growth. The diploid number in channel catfish appears to be in the 52 to 56 range.

An infection of channel catfish---An undiagnosed "infection" resulted in mortality of large numbers of our fingerling channel catfish and all of the fingerling flathead catfish last summer. Unless the condition was checked by an appropriate treatment, all the fish would be dead within 7 to 10 days.

After reviewing the literature and the treatment of similar diseases of cold-water fishes, we planned a treatment of 2 grams of terramycin in 100 grams of feed. The feed, fresh beef liver, was pulverized in a blender to which was added a complete vitamin supplement and the terramycin. After this mixture was properly blended, an amount of CMC to slightly "thicken" the mixture was added. This mixture was frozen until used.

Within 12 hours after the initial feeding of the vitamin-supplemented-terramycin beef liver, mortality ceased. This treatment was continued for 3 - 5 days after which plain beef liver and dry feeds were fed. Usually one treatment period was sufficient and the infection did not reappear.

New laboratory facilities---The new office-laboratory and service buildings were occupied in December, 1963. Most of the month of December was devoted to vacating the previous facilities and arranging the equipment and materials in the new facilities.

#### Papers in manuscript

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Beaver, John A. and Kermit E. Sneed.  
Comparison of growth rate of male and female channel catfish, Ictalurus punctatus .

Busch, Robert E., Kermit E. Sneed, and Harry K. Dupree. Use of hormone-cholesterol pellets to control gonadal development of goldfish, Carassius auratus .

Dupree, Harry K.  
Water-soluble vitamins essential for the growth of channel catfish, Ictalurus punctatus

Dupree, Harry K. and Kermit E. Sneed.  
Purified diets for channel catfish nutritional research.

Trandahl, Arden J. and Harry K. Dupree.  
Effect of diet, environment, and disease on the electrophoretic blood protein patterns of fourteen groups of channel catfish, Ictalurus punctatus .

#### STAFF

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Dr. Harry K. Dupree, Fishery Biologist  
Mr. Ortus L. Green, Fish Hatchery Manager  
Dr. Robert E. Busch, Geneticist

Mrs. Mabel A. Jones, Clerk-Stenographer  
Mrs. Clara B. A. Daniel, Biological Laboratory Aid  
Mr. Eugene McCauley, Fish Hatcheryman

## PESTICIDES

### FISH-PESTICIDE RESEARCH LABORATORY

Denver, Colorado; Jackson, Wyoming; Tishomingo, Oklahoma; Patuxent, Maryland; Marion, Alabama  
Oliver B. Cope, Chief



Fish-Pesticide Research Laboratory, Building 45, Denver Federal Center

#### HIGHLIGHTS

Cutthroat trout given periodic treatments of malathion in feed and by contact at Jackson showed brain cholinesterase inhibition after every exposure, with recovery to levels below control levels after 35-day intervals.

Bluegills exposed to sodium arsenite in pond tests at La Crosse, Wisconsin accumulated arsenic residues proportional to amount of exposure. Growth and survival of immature bluegills in the ponds was inversely proportional to amount of exposure.

Adult redear sunfish exposed to Kuron at Durant, Oklahoma developed pathological conditions in livers and testes.

Bluegills fed heptachlor in pond tests at Marion, Alabama showed better growth in control and low-treatment tests than in high dosage tests.

Rainbow trout fed various diets for 26 months in nine National Fish Hatcheries were analyzed for pesticide residues. All fish analyzed contained DDT and its products, and some diets appeared to induce larger residues than did others.

Influence of time and temperature on toxicity of DDT and toxaphene to bluegills was measured at Denver. Toxaphene followed the usual pattern, but DDT was more toxic at low temperatures than at high ones, with levelling off at 45° and 85°F.

The effects of water hardness on toxicity of silvex to sunfish at Patuxent, Maryland were measured; a trend toward higher toxicity with increased water hardness appears.

Various toxicity measurements are reported for previously untested pesticides and fish species.

Toxicity tests performed with insecticides against six kinds of aquatic insects are reported.

A study of a micro-environment treated with C<sup>14</sup>-labelled DDT revealed rapid and great accumulation of DDT in and on aquatic vegetation. Bluegills took up the insecticide at a faster rate than snails.

### PEST CONTROL PROGRAMS

A grasshopper control program was carried on by the U. S. Department of Agriculture on the Boise National Forest in July. Malathion was sprayed from airplanes at the rate of 3/4 pound per acre on 65,000 acres. Our staff studied short-term effects in the water at strategic sites. Observations on resident fish in the streams and on hatchery trout placed in live-cars indicated no mortality or other adverse effects on fish. Brain cholinesterase activity in rainbow trout in live-cars was slightly reduced in some cases, but were at normal levels within 15 days. Aquatic insects were killed or immobilized by the spray on at least two small tributaries of the Boise River, but many apparently healthy insects remained in the stream after the dissipation of the malathion.

### EXPERIMENTAL FIELD STUDIES

#### Malathion and cutthroat trout at Jackson, Wyoming

The long-term experiment dealing with chronic effects of malathion on cutthroat trout began at Jackson, Wyoming. Measurements were made on brain cholinesterase activity, growth, mortality, and histopathological changes in the first phase of the work, in which lots of fish were given malathion in bath form every 35 days; other lots were given malathion in their feed, also at 35 day intervals; the control lot was given no malathion. The second phase, which will also consider reproductive success, will begin in January, 1964.

Cumulative mortality in both lots of bathed fish exceeded that of the control lot throughout most of the year. Mortality in the fed lots approximated that of the control lot.

The number of dead trout exhibiting traumatic injury or disease symptoms was about the same in all lots. This is in contrast to what happened in DDT experiments, where disease symptoms were observed more often in dead fish in low-dosage lots than in high-dosage lots.

Hematocrit values decreased in all lots from January through September. There was no consistent pattern of hematocrit values from lot to lot; hematocrit values did not seem to be related to amount of exposure to malathion.

Consistent relationships appeared among lots of trout with respect to inhibition and recovery of brain cholinesterase levels. Highest activity was always seen in control fish, and lowest activity in the high-treated bathed fish. Patterns of recovery between treatments were fairly uniform. Figure 1 shows brain cholinesterase measurements for lot I, the control lot, lot II, the lot bathed at 1.0 ppm, and lot IV, the lot fed 8.0 mg/k. in the feed.

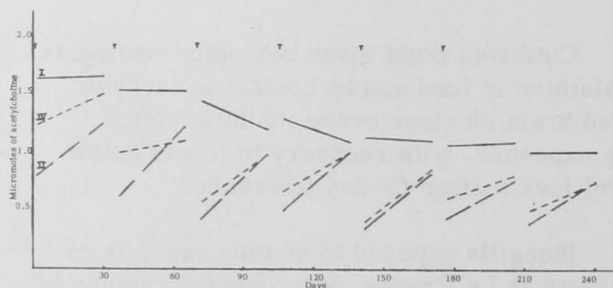


Figure 1:--Effects of periodic exposure of three lots of cutthroat trout to malathion. Enzyme activity expressed as micromoles of acetylcholine hydrolyzed per 2 mg. of brain tissue in 30 minutes at 25° C. Treatments given at times shown by "T". Lot I was the control group. Lot II was bathed in malathion solution. Lot IV was given malathion in feed.

Sodium arsenite and bluegills at La Crosse, Wisconsin

In 1963, considerable work was done in the laboratory to analyze samples of water, bluegills, and soils exposed in outdoor plastic pools in the 1962 experiment. In addition, a new chronic effect experiment was carried on in 1963, with many measurements made and samples preserved for chemical analysis during the winter.

The amount of arsenic in water samples collected in 1962 was proportional to the amount of sodium arsenite applied to the pools. The more frequent the application the greater the amount of chemical in the samples. The arsenic content of fish collected in 1962 was generally proportional to the strength of the exposures. In soil samples taken at eight weeks there was more arsenic from pools treated once than from pools treated with more total arsenic at weekly intervals. Table 1 shows these results.

Results available now from the 1963 exposures show that microhematocrit measurements were not correlated with concentrations of sodium arsenite in the pools. The ratio of gonad weight to body weight in bluegills collected after 8 and 16 weeks showed no differences directly attributable to amount of exposure to sodium arsenite. Survival of young-of-the-year fish after 16 weeks seemed to be influenced by degree of exposure to the chemical; survival was highest in the control and lowest in groups receiving 1.2 ppm at weekly and monthly intervals. The survival of adults, however, was not proportional to strength of treatment. Table 2 shows survival measurements for immatures and adults.

Growth in length and weight of young-of-the-year bluegills tended to be inversely proportional to concentration of sodium arsenite in the pools, as seen in Table 3.

Adult bluegills in general sustained losses in weight, probably due to a failure of the natural food supply. The greatest losses in weight occurred in pools with the highest concentrations of arsenite in each treatment series.

Pathological examination of exposed fish from the ponds in 1963 revealed that few changes took place in the first few weeks. After that, some startling changes were apparent. No nematodes were found in the pyloric caecae, in contrast to the earlier condition. Kidney and liver appeared in the later samples. There is also a possibility of degenerative lesions in the ovaries of some fish.

Table 1:-- Arsenic residues in water, bottom soils, and fish from pools treated for an 8-week period in 1962.

Pool No.	Herbicide application rate (ppm As <sup>3</sup> )	Residues at end of 8 weeks (ppm As <sup>3</sup> )		
		Water	Fish Flesh	Soil
1	one/year 2.31	1.01	0.38	92.1
2	one/year 0.69	0.056	0.35	37.3
3	one/year 0.23	0.024	1.02 <sup>1/</sup>	10.7
4	every month 0.69	0.43	0.17	38.1
5	every month 0.23	0.12	0.20	22.5
6	control	ND <sup>2/</sup>	ND	ND
7	every week 0.69	4.81	3.88 <sup>1/</sup>	44.9
8	every week 0.23	0.98	0.78 <sup>1/</sup>	36.7
9	every week 0.023	0.12	0.09	6.5

1/ Small fish

2/ ND denotes no detectable amount

Table 2:-- Survival of immature and adult bluegills after a 16-week exposure to various concentrations of sodium arsenite in plastic pools in 1963.

Pool No.	Herbicide application rate (ppm As <sup>3</sup> )	Young-of-year		Adult		
		Number stocked	Number surviving	Number after sampling	Number surviving	Percent surviving
1	one/year 4.0	200	103	154	95	62
2	one/year 1.2	200	108	154	89	58
3	one/year 0.4	200	159	154	80	52
4	every month 1.2	200	90	134	81	60
5	every month 0.4	200	163	151	78	52
6	control	200	179	133	80	60
7	every week 1.2	200	35	143	44	31
8	every week 0.4	200	145	163	82	50
9	every week 0.04	200	147	163	89	55



Table 3:-- Growth of young-of-the-year bluegills over a 16-week exposure to various concentrations of sodium arsenite in plastic pools in 1963.

Pool No.	Herbicide application rate (ppm As <sup>3</sup> )	Average length (in.)		Average weight (gm.)		Weight ratio, final/orig.
		Orig.	After 16 weeks exposure	Orig.	After 16 weeks exposure	
1	one/year 4.0	1.37	1.98	0.54	1.92	3.5
2	one/year 1.2	1.37	2.42	0.54	3.93	7.3
3	one/year 0.4	1.37	2.57	0.54	4.48	8.3
4	every month 1.2	1.37	2.06	0.54	1.76	3.3
5	every month 0.4	1.37	2.53	0.54	4.62	8.6
6	control	1.37	2.40	0.54	3.32	6.1
7	every week 1.2	1.37	1.93	0.54	1.53	2.8
8	every week 0.4	1.37	2.27	0.54	2.88	5.3
9	every week 0.04	1.37	2.48	0.54	4.21	7.8

### Herbicides and sunfishes, Oklahoma

Pond tests at Durant, Oklahoma were run with Kuron (propylene glycol butyl ether esters of 2,4,5-TP) to measure chronic effects on redear sunfish. Four ponds were used, one being an untreated control and others receiving 1, 3, and 10 ppm, respectively. Applications of Kuron were made on March 21, April 20, and May 22. The experiment was terminated on June 14.

Residues of Kuron in the ponds increased with time, degradation of the herbicide proceeding at slower rates than the addition of new chemical under the schedule. The result was the development of residues in the water to levels greatly exceeding what would be used in aquatic weed control. Some apparent effects on the fish were measured. The average sizes of fish taken in trap samples had greater lengths and weights in the ponds that were treated than in the control pond, and the stronger the treatment, the larger and heavier were the fish. Microhematocrit measurements were higher in treated fish than in untreated fish, but the heavily-treated fish did not have higher hematocrits than those exposed to lighter treatments. Definite pathology was found associated with exposure to Kuron. Well defined liver degenerative lesions were found from 2 weeks on in the 3- and 10 ppm-treated fish, but not at any time in the control

or the group treated at 1 ppm. From the 5th week testicular degenerative lesions were seen in the 3 and 10 ppm groups, resulting in apparent exhaustion atrophy of the spermatid tubules and production of immature, atypical, and abnormal spermatozoa. No comparable changes were seen in ovaries.

Experiments in ponds at Tishomingo, Oklahoma were begun in September to measure chronic effects of Kurosol SL (potassium salt of 2,4,5-TP) on bluegills. Applications were made to ponds at 75, 25, 10, and 0 ppm. There was no immediate effect on the fish, but filamentous algae and *Potamogeton* began to die at the outset. *Nais* was eliminated after three weeks, but *Chara* was not affected. Fish died in limited numbers in the 75 ppm ponds after a few weeks, and about 35 percent of the fish in these ponds had died after 2-1/2 months. Mortality in the 25 ppm ponds took place at lower rates, but none has been seen in the 10 ppm ponds or in the control ponds.

Kurosol in the pond water has not been as persistent as was the Kuron. The Kurosol broke down at faster rates than the repeated additions to the ponds once a month.

### Heptachlor and bluegills at Marion, Alabama

Two outdoor experiments were carried on with the chlorinated hydrocarbon, heptachlor, and bluegills at Marion, Alabama to measure chronic effects on the fish. One study featured the feeding of heptachlor at various levels in the diet to bluegills in plastic wading pools, and the other study took place in 1/10-acre earthen ponds to which bluegills and heptachlor had been added.

The pond study, with exposure at 0.05, 0.0375, 0.025, 0.0125, and 0 ppm of heptachlor, showed rapid development of residues of heptachlor, heptachlor epoxide, and related compounds in pond water, bottom sediments, and fish. Build-up of residues in fish and water was immediate, but no residues were found in bottom sediments until after the 14-day sample. Heptachlor measured in water never approached the amounts of toxicant added. Residues in soils were found only in the two highest levels of

treatment, never exceeded by much the amounts added to the ponds, and were not found after the 56-day sample, in June. Table 4 summarizes the residue data for fish.

Pathological examination of 99 fish collected through 28 days in these tests revealed no tissue or cell changes associated with exposure to heptachlor.

Growth of fish in the ponds, as indicated by average sizes of fish taken in samples throughout the experiment, was different from pond to pond. In general, the average size of fish taken from heavily treated ponds was greater than in fish from the control and lightly-treated ponds.

The plastic pool study, with feeding of heptachlor at 25, 10, 5, and 0 mg/kg, showed development of residues of heptachlor, heptachlor epoxide, and related compounds in the bluegill whole bodies. Residues did not form as fast or to as great an extent as in the pond studies described above. Also, as in the pond studies, DDT and its products appeared in many fish; in this experiment the appearance was in the 112-day sample in October.

No heptachlor was found in bluegills after the 56-day sample. A fact not explained is the appearance of DDT and its degradation products in fish at about the time of the disappearance of the heptachlor. DDT was not found in water, mud, or vegetation, and the source of the DDT in the fish is not known.

Numbers of invertebrates sampled from the ponds by traps and with dredges indicated that the control pond had greater numbers of organisms, exclusive of the gastropods, than did the treated ponds during most of the experiment. Greatest numbers of invertebrates were generally trapped at the 24-inch depth.

Table 4:--Total residues, in ppm, of heptachlor, heptachlor epoxide, and related compounds in whole bodies of bluegills exposed to one application in ponds at Marion, Alabama, beginning in April, 1963.

Time after treatment	Pond 1 Treated at 0.05 ppm	Pond 2 Treated at 0.0375 ppm	Pond 3 Control	Pond 4 Treated at 0.025ppm	Pond 5 Treated at 0.0125 ppm
16 hours	9.2	5.7	—	—	—
1 day	28.7	27.2	ND <sup>1/</sup>	—	11.1
3 days	27.7	45.1	ND	4.0	3.8
7 "	52.6	29.5	ND	9.4	0.9
15 "	56.8	19.8	ND	11.1	3.2
28 "	15.7	8.1	ND	8.0	0.5
56 "	0.15	0.18	ND	0.15	—
84 "	ND	ND	ND	ND	ND
125 "	ND	ND	ND	ND	ND
140 "	ND	ND	ND	ND	ND

<sup>1/</sup> ND denotes no detectable amount

Table 5:--Average weights of bluegills on August 30 after receiving heptachlor in feed for three months.

Heptachlor concentration in feed, mg/kg.	Number of fish sampled	Total weight of samples, g.	Average weight per fish, g.
25	15	93	6.20
10	37	316	8.54
5	69	681	9.87
Control	48	573	11.94

These results do not agree with those derived from the pond study described above, but differences in sampling methods and treatment rates may explain the discrepancy.

Residues in fish from hatcheries

Rainbow trout fed for 26 months on various diets in a large-scale hepatoma induction experiment were submitted to this laboratory from nine National Fish Hatcheries in different sections of the United States. Chemical analyses were made of whole bodies of the fish, and the only pesticide found in any fish was the

DDT complex. DDT and its metabolites were found in every fish examined, and there was variation from sample to sample, from station to station, and from diet to diet. We conclude that the greatest cause of variation in DDT residues was differences in diets. Table 6 summarizes the findings.

Table 6:--Total amounts of DDT and its products measured in rainbow trout from nine hatcheries and fed various diets for 26 months. DDT content expressed as ppm., each number representing one sample.

Diet	McNary	Norfolk	Leetown	Hagerman	Lamar	Quilcene	Ennis	Manchester	Spearfish
Clark	0.66	1.05	0.58	0.69	0.72	1.22	1.30	0.65	1.18
		0.56	0.88			0.75	0.85	0.80	1.21
Rangen	0.62	0.86	0.97	0.97	0.59	0.71	0.59	0.47	0.37
		0.92	0.34		0.82	0.50	0.56	0.45	0.50
Glencoe	-	0.34	-	-	-	-	-	-	0.27
		0.34							0.12
Strike	-	0.71	-	-	0.62	-	-	-	-
		0.58			0.44				
Purina	0.18	-	0.16 (20%)	0.25	-	-	-	0.37	-
								0.37	
Hill	-	-	-	0.33	-	-	-	-	-
Murray	0.13	-	-	0.13	-	0.10	0.19	-	-
						0.23	0.17		
Stockton	-	-	-	0.12	-	-	-	-	-
Small's Dina Fish	-	-	-	0.88	-	0.36	0.82	-	-
						0.77	1.08		
Cortland #6	-	-	-	-	0.80	-	-	-	-
					0.69				
Oregon Moist Pellet	-	-	-	0.60	-	-	-	-	-
100% Meat	-	-	0.07	-	-	-	-	0.11	0.25 (liver)
			0.10					0.09	0.18

## LABORATORY STUDIES

### Fish toxicity tests at Denver

Toxicity tests at Denver included time-temperature studies, preliminary bioassay tests on new pesticides or different species of fish, and comparisons of toxicities of pyrethrum and rotenone.

1. The toxicity of DDT and toxaphene to bluegills was determined at various temperatures and times of exposure (table 7). In general, the toxicity of DDT increased with a decrease in temperature, with the effect of temperature tending to level off at 45° and 85°. The 25 hour LC<sub>50</sub> at 45° appears somewhat high. In this test fish were completely immobilized at concentrations one-third to one-fourth of the LC<sub>50</sub> values, but they were not dead. The toxicity of toxaphene increases moderately with increase in temperature. The inverse relationship seen with DDT agrees with toxicity-temperature relationships seen in insects.

2. The toxicity of similar emulsifiable formulations of rotenone and pyrethrum to rainbow trout, channel catfish, and bluegills are presented in table 8. It is clear that the rotenone formulation is more toxic to these species than is the pyrethrum formulation.

3. Results of different bioassay tests made during the year with miscellaneous insecticides and herbicides are reported in table 9.

### Fish toxicity tests at Patuxent

Bioassay work was carried on at the Patuxent Wildlife Research Center after the new laboratory was put into operation in 1963. The work at Patuxent consisted primarily of testing of herbicides against fish, with emphasis on the effects of water hardness on toxicity.

Table 10 shows some toxicity measurements with silvex and bluegill and pygmy sunfish. Trends toward higher toxicity in harder waters are seen, but the differences in toxicity are not statistically significant.

Table 7:--Influence of time and temperature on toxicity of two insecticides to bluegills averaging 1.2 grams in weight.

Temperature, °F.	LC <sub>50</sub> in µg./l.		
	24 Hr.	48 Hr.	96 Hr.
DDT and bluegills			
45	21.	2.4	1.6
55	5.0	2.6	2.0
65	7.4	5.4	4.5
75	9.2	7.0	6.6
85	9.8	6.4	5.6
Toxaphene and bluegills			
45	35.	9.6	4.3
55	9.6	5.5	3.4
65	7.2	4.9	2.6
75	6.6(7.0) <sup>1/</sup>	3.8(6.0) <sup>1/</sup>	2.5(5.0) <sup>1/</sup>

<sup>1/</sup> Figures in parentheses are LC<sub>50</sub> values for DDT for this lot of fish.

Table 8:--Toxicity of rotenone and pyrethrum formulations<sup>1/</sup> to three species of fish

Exposure period	LC <sub>50</sub> in µg. of total material/l.	
	Pyrethrum	Rotenone
Rainbow trout (0.3 gram) Tested at 55°F.		
24 Hrs.	56	32(3.0) <sup>2/</sup>
48 "	54	28(2.0)
96 "	54	26(1.5)
Channel catfish (0.5 gram) Tested at 75°F.		
24 Hrs.	96	32(4.9)
48 "	82	29(4.1)
96 "	78	26(3.3)
Bluegills(0.6 gram) Tested at 75°F.		
24 Hrs.	78	24(8.4)
48 "	70	22(6.0)
96 "	70	22(4.7)

<sup>1/</sup> Rotenone 0.7% active ingredient; pyrethrum 1.0% active ingredient.

Same materials used to prepare both formulations, except that the rotenone preparation contained 6% more xylene than did the pyrethrum.

<sup>2/</sup> Figures in parentheses are LC<sub>50</sub> values for DDT in µg./l. for the lot of fish tested.

Table 9:--Toxicity measurements of various pesticides versus fish.

Toxicant	Species	Wt. or length	Temp °F.	Estimated LC <sub>50</sub> , µ/l.		
				24 hr.	48 hr.	96 hr.
Aldrin, tech	Black bullhead	1.5g	75	22	19	19
Apholate, tech. <sup>1/</sup>	Rainbow	1.5g	55	Not affected at 40,000 µ/l. for 96 hours.		
DDT, p,p	Black bullhead	0.9g	75	65	47	27
DDT, p,p	Channel catfish	1.4g	75	4.2	3.3	2.9
Dieldrin, tech	Black bullhead	1.5g	75	11	10	10
Dimethoate, tech	Rainbow	1.5g	55	20,000	12,000	8,500
Endrin, tech	Black bullhead	1.5g	75	1.3	1.1	1.1
	Channel catfish	1.4g	75	0.45	0.34	0.29
Ethyl Guthion, tech	Bluegill	0.8g	75	3.8	1.4	-
Heptachlor, tech	Black bullhead	0.9g	75	76	50	34
Lindane, tech	Bluegill	0.8g	75	61	26	-
	Rainbow	5.4g	55	49	-	-
Toxaphene, tech	Black bullhead	0.9g	75	7.7	6.4	5.8
PGE esters of:						
2,4-D, E.C. 70.5%	Rainbow	1.5g	55	1,200	1,100	1,100
2,4-D, Tech. 100%	Rainbow	1.5g	55	1,200	1,100	1,100
2,4,5-TP, E.C. 65.5%	Rainbow	1.5g	55	750	650	600
2,4,5-TP, Tech 100%	Rainbow	1.5g	55	1,500	1,400	1,300

<sup>1/</sup> Insect sterilant

Table 10:--Toxicity of butoxyethanol ester of 2,4,5-TP to two species of fish, in waters of various conductivities and pH values.

Conductivity, micromhos	pH	LC <sub>50</sub> in mg./l		
		24 hrs.	48 hrs.	96 hrs.
<u>Bluegill, 0.75g</u>				
101.6	7.4	.460	.415	.405
171.2	8.1	.575	.520	.500
176.4	7.4	.440	.395	.390
404.3	8.1	.545	.530	.520
439.6	7.4	.450	.432	.418
650.5	7.8	.565	.550	.535
654.0	6.1	.400	.360	-
<u>Pygmy sunfish</u>				
82.2	5.3	.763	.758	.720
155	5.5	.738	.699	.633
408.4	4.8	.603	.532	.489
646.5	6.3	.635	.590	.580



## Insect toxicity tests at Denver

Bioassay tests were conducted on wild aquatic insects collected in the field, held in the laboratory, and tested under standardized

conditions. Table 11 summarizes the results of tests involving six species of insects and nine insecticides, and shows that toxicity to these insects does not correspond to toxicities to fish.

Table 11:--Toxicity of various insecticides to some immature aquatic insects, tested at 60<sup>o</sup>F.

Genus and Toxicant	LC <sub>50</sub> in ug./l.		
	24 Hr.	48 Hr.	96 Hr.
<u>Pteronarcys</u> (stonefly)			
Aldrin	30.0	8.0	1.3
DDT	45.0	16.0	9.0
Dibrom	27.0	16.0	8.0
Dieldrin	6.0	1.1	0.70
Endrin	4.0	0.96	0.25
Heptachlor	8.0	5.6	1.1
Lindane	1.2	1.0	—
Malathion	40.0	20.0	10.0
Phosphamidon	1400.	460.	130.
Toxaphene	18.	7.0	2.3
<u>Pteronarcella</u> (stonefly)			
DDT	—	5.0	3.0
Malathion	12.0	6.0	—
<u>Heptagenia</u> (mayfly)			
DDT	0.6	0.3	0.1
Malathion	0.4	0.2	—
<u>Lestes</u> (damselfly)			
DDT	30.0	10.0	—
<u>Hydropsyche</u> (caddisfly)			
DDT	7.4	3.4	1.7
Malathion	26.	13.	5.0
Toxaphene	33.	27.	4.2
<u>Atherix</u> (snipefly)			
DDT	84.	25.	17.

## C<sup>14</sup> DDT in a microenvironment at Denver

Six hundred micrograms of C<sup>14</sup>-labelled DDT was placed in 30 liters of water in each of four aquaria, together with soil and aquatic vegetation. Small bluegills were added to two of the aquaria after 28 days, and snails of the genus *Ampullaria* after 6 weeks. The objectives of the study were to measure the breakdown of DDT in parts of the system and to learn if DDT would return to the water from high-residue components of the environment after the toxicant had reached near-zero levels in the water.

Samples of water, soil, and vegetation were taken from all aquaria for analysis. The vegetation was consumed by the snails in the two aquaria where they were present. Vegetation samples taken after that time were from the other two aquaria, and the snails subsisted on lettuce fed every two days.

Fourteen days after the addition of the DDT, the level in the water was down to 0.42 ppb, was 6.0 ppb in the soil, and had reached 15,600 ppb in the vegetation. At 4 weeks when the fish were added, the water contained 0.30 ppb. The fish accumulated residues to more than 1,000 ppb in a week and a half, while the amounts in the mud were decreasing and those in the vegetation were still increasing. When the snails were added at 6 weeks, the water had 0.19 ppb, the soil had 1.1 ppb, the vegetation had 23,400 ppb, and the fish about 1,000 ppb. Two weeks later, the water was reduced to 0.08 ppb, the vegetation reduced to 20,700 ppb, and the snails contained 160 ppb. At 15 weeks, all parts of the environment still contained some DDT, but declines were apparent. Had the snails eaten vegetation containing DDT from the 8th to 15th week, the decline in DDT levels in the snails might not have occurred. Table 12 summarizes the residue measurements made in the study.

## Guppies and apholate at Denver

This experiment involved the exposure of guppies to sublethal levels of apholate, an insect sterilant, to measure chronic effects. The exposure phase was terminated at the end of the year, and the remaining adult fish were

preserved for histopathological examination. A report will be prepared when all data have been analyzed.

Table 12:--Total amounts of DDT and its metabolites measured in components of the micro-environment. 20  $\mu$ l of C<sup>14</sup>-labelled DDT placed in the system in one application.

Time elapsed	Total residues, $\mu$ /l				
	Water	Soil	Vegetation	Fish	Snails
10 days	1.1	-	-		
2 weeks	0.42	6.0	15,600		
3 "	0.07	3.5	19,800		
4 "	0.30	-	-	1/	
6 "	0.19	1.1	23,400	Approx. 1000	2/
8 "	0.08	-	20,700	-	160
11 "	0.06	-	9,500	-	120
15 "	0.03	-	4,100	-	140
21 "	0.03	-	1,960	-	28

1/ Bluegills were added to the system at 4 weeks. 3-day samples averaged 270 ppb.

2/ Snails were added to the system at 6 weeks. 4-day sample measured 140 ppb.

## Chemistry methods

Methods for the determination of Kuron and Mirex in fish and water have been partially developed.

The procedure for Kuron in fish consists of extraction with 10 percent ethyl ether in petroleum ether, followed by partitioning of the extract residue between hexane and acetonitrile, elution through a column of acid-washed chromatographic adsorption alumina, and final estimation by paper chromatography using silver nitrate spray to develop the spots of Kuron. Early data indicate a recovery of 54 percent. The paper chromatographic development will afford detection and estimation of as little as 0.1  $\mu$ g. on the paper.

Mirex determination in fish consists of extraction with 10 percent ethyl ether in petroleum ether, passage of the extract residue through an MgO celite column, treatment with fuming sulfuric acid, and paper chromatography-silver nitrate development. Early data indicate 83 percent recovery with sensitivity to 0.1  $\mu$ g. on the paper.

Kuron and Mirex can be recovered from water by adsorption on activated charcoal. The recoveries are 49 and 84 percent, respectively.

Tests have been made to compare the efficiencies of different activated charcoals used in the analysis of DDT in water samples. Those tested were Nuchar C-190, +30 mesh, coconut charcoal, 6-14 mesh, and coconut char-

coal, powdered. The granular and powdered coconut charcoals are roughly equivalent in their ability to absorb DDT from water. They are inferior in this respect to the Nuchar C-190, but they contribute less contaminating material which interferes in the chromatographic development. The recovery factor for Nuchar C-190 is 70 percent in the range of 10-20  $\mu\text{g}$ . DDT per gallon of water. The factor for granular coconut charcoal is 40 percent.

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## CONTROL

FISH CONTROL LABORATORIES  
La Crosse, Wisconsin; Warm Springs, Georgia  
Robert E. Lennon, Chief



Fish Control Laboratory, La Crosse, Wisconsin

### HIGHLIGHTS

Fifty-one cooperators contributed 521 chemicals for screening. Good progress was made in the bioassay program, but the quantity and quality of compounds available were limited as an unfortunate result of the highly-publicized problems with pesticides and certain pharmaceuticals.

Most of the compounds used were carefully selected according to known or suspected biological activities. This approach yielded good numbers of chemicals which deserved further investigation. Eighty-five chemicals were advanced into Delineative Screening and 30 in Intensive Screening. In addition, 45 mixtures of 9 compounds were evaluated for synergism or other potentiation.

Studies in the laboratory and field were concentrated on an antibiotic which exhibits fine

potential as a fish toxicant. Twenty-five species of fish were killed in the laboratory at concentrations of 0.1 to 120 ppb. In limited field trials carp and goldfish were killed at 10 ppb. No significant harm was done to bottom fauna and amphibians.

The Fish Control Laboratory at Warm Springs, Georgia, was activated. Some buildings are completed; others are nearly completed. A staff was recruited and trained at La Crosse, furnishings and equipment were purchased, and plans for remaining construction were prepared. Limited investigations in fish control will begin early in 1964.

### CHEMICAL SCREENING

The screening of chemicals for possible fish control agents proceeded well along a broad front at the La Crosse Laboratory during the year. Fifty-one cooperators submitted 521

compounds. As great a selection of chemicals as possible was subjected to screening to determine classes which appear to hold the most promise for future investigation. Thus a background of information is established which will enable us to better move ahead with studies on chosen groups during the coming year,

Some circumstances made the 1963 screening program atypical in important respects. First, the unfortunate situations with thalidomide and lysergic acids followed by Miss Carson's "Silent Spring" caused segments of the chemical industry to curb contributions of research compounds to investigators. This curtailment may prevail until Congress and State legislatures have considered and acted on new proposals concerning pesticides and pharmaceuticals. Second, an agency of the Department is involved in a patent dispute with a chemical company. The chemical industry is watching the case closely, and while awaiting a court decision many companies will not contribute "unknown" chemicals for testing.

The patent dispute has strengthened also a contention of some chemical manufacturers that contracts for screening must be negotiated between them and investigators which would guarantee protection and some unusual advantages for the companies. We have encountered refusals to furnish chemicals until contracts are in effect. On the other hand, some proposed contracts are not acceptable to the Bureau. Efforts are in progress in the Bureau and Department to draft general contract provisions which will be acceptable to the companies and the government.

We are looking forward to an amelioration of these circumstances. Many of the compounds or classes of compounds which we would like to have tested in 1963 were not obtainable. Moreover, the usually fruitful contracts with the industry's research people largely failed to compensate. We found that some important contributions planned for us by industrial chemists were prohibited by company legal divisions.

A larger than usual volume of screening was made possible during the second half of the year by assignment of Warm Springs trainees at

La Crosse. Two fishery biologists were on hand for two months, and two chemists remained for six months. They worked in all phases of the bioassay in the laboratory, plastic pools, and field.

A wide variety of fish of different life stages was used not only to better check the performance of test chemicals but to determine which fish can be best employed and obtained in the quantities, sizes, and condition desired. The Branch of Fish Hatcheries held a seminar of fish culturists at La Crosse in March, and as a result a fine plan of culturing and supplying research fishes was defined. The National Fish Hatcheries gave us splendid service in every respect. In addition, the Wisconsin Conservation Department furnished nearly 300 pounds of fish for a field trial of a toxicant. In all, approximately 498,000 fish of 34 species were acquired for tests in the laboratory and field. Also, 10,000 rainbow trout eggs and 5,000 sac-fry brook trout were obtained for special bioassays.

#### Preliminary screening

Four-hundred-thirty chemicals including pesticides, biologicals, pharmaceuticals, and miscellaneous or coded compounds were tested at 0.01, 0.1, 1.0, and 10 ppm in static bioassays against rainbow trout, goldfish, black bullhead, and bluegill in reconstituted water at 12° C. Of them, 47 pesticides, 5 pharmaceuticals, and 12 miscellaneous or coded compounds displayed biological activities which deserve further investigation. None of the biologicals showed promise against fish.

The activities observed were primarily general or selective, acute toxicities. Although every opportunity is taken to choose compounds for screening on the basis of known or suspected properties, we were faced this year with relatively few opportunities to do so because of reasons given in the introductory remarks. Thus, many of the compounds were screened "blind" and this becomes largely a matter of detecting acute toxicities.

The bioassay procedures were thoroughly reviewed and evaluated early in the year. Modifications were made to economize on glass jars,



to lessen time and labor in arranging tests, and to facilitate the recording of test results. New systems were inaugurated for assigning accession numbers to compounds, for inventory and identification of chemicals, for retrieval of data, and for reporting the results of screening to contributors. Methods for statistical analysis of data were tested and adopted. We reviewed patent problems and procedures with a Department solicitor, with representatives of the National Agricultural Chemicals Association, with BCF Sea Lamprey Investigations, and with the Regional Office.

The retrieval of data remains a problem and grows worse with the increasing volume and diversity of results. The possibilities of machine aids are under consideration.

On advice from a Department solicitor and industry representatives, steps were taken to provide the elaborate security desired by companies on chemicals, especially non-patented or non-registered items, submitted to us for screening. Furthermore, we elected to follow suggestions that test compounds be identified in reports of progress by company or Laboratory code numbers only.

#### Delineative screening

Eighty-five selected compounds were tested at 12° C. against rainbow trout, goldfish, black bullhead, and bluegill sunfish to find the concentrations which evoked all-or-none responses in the fish. Thirty-two which included mostly pesticides gave favorable results. On the basis of a chemical's characteristics and performance at threshold concentrations and on estimates of its applicability and market as a fish control agent, a decision is made whether to test it further or abandon it.

The definition of threshold or effective concentrations of a chemical is a lengthy and laborious process. The bracketing and pinpointing of concentrations which produce all-or-none responses at a single temperature typically require 528 fish, 264 1-gallon jars, 44 aliquots of test solutions, and 1,320 observations for the tests, controls, and replicates.

The effective concentrations are confirmed, for example, by at least three replicates.

Pesticides:--The usual order of susceptibility to pesticides in our screening is: rainbow trout, bluegill, black bullhead, and goldfish. Both the order and the range of susceptibilities within it are helpful in detecting desirable properties of chemicals. The following are good examples.

Compound 139 produced the usual order of toxicity and was effective on the four species at less than 0.4 ppm. Compound 137 demonstrated the same order but killed the trout and bluegill at 1/100,000 of the concentration necessary to kill bullhead and goldfish. Others, however, gave results which differed from the usual order. Three chemicals killed bluegills at concentrations in which the other species survived. Four caused death to bullhead in quantities which enabled bluegill to live. Compound 412 was more interesting because it killed goldfish at concentrations which were no serious threat to the other three species.

Pharmaceuticals:--Two of the six compounds selected demonstrated toxicities of interest. Compound 471 killed all fish at 0.4 to 1.5 ppm. Compound 184 killed rainbow trout and black bullhead at less than 1 ppm, goldfish at 3 ppm, and bluegill at 10 ppm.

Miscellaneous or coded compounds:--Six of 12 chemicals showed potentials for fish control. One caused death of all fish at 0.5 ppm. The others varied from the usual order of toxicity. Compound 168 killed goldfish and bluegill at 0.4 ppm, black bullhead at 0.7, and rainbow trout at 1 ppm. A wider range of toxicity was exhibited by compound 255; bluegill died at 0.8 ppm, rainbow trout and goldfish at 3 ppm, and bullhead at 8 ppm. Bluegill survived concentrations of compound 169 which killed the other species.

The Delineative program includes re-screening of promising compounds at 17 and 22° C. to detect the influences of temperature on effective concentrations. These steps are optional, however, and may be postponed until Intensive Screening if previous results warrant.

## Intensive screening

Thirty chemicals were subjected to advanced bioassays at 12, 17, and 22° C. with gizzard shad, brown trout, brook trout, northern pike, carp, white sucker, yellow bullhead, channel catfish, green sunfish, pumpkinseed, longear sunfish, Iowa darter, yellow perch, and walleye. Some tests also involved life history stages of certain fish including egg, sac-fry, fingerling, and adult.

The most advanced bioassays were expanded to include *Daphnia magna*, mayfly nymphs, damselfly nymphs, crayfish, tadpoles, and salamanders. Procedures for the acquisition, holding, and use of these animals were investigated.

Organic phosphates:--Compounds of this class continue to demonstrate far greater toxicity to sunfishes than to other species. Some show increasing potential as aids to fish harvest, fish thinners, and general control in the culture of bait minnows, catfishes, and other pond species. Experiments on potentiation of organic phosphates with halogenated insecticides show high degrees of selective toxicity to certain sunfish at low concentrations.

Chlorinated hydrocarbons:--Endrin, aldrin, dieldrin, thiodan, toxaphene, and chlordane were tested against ten species at three temperatures. Endrin and thiodan were the most toxic, and northern pike were the most sensitive and succumbed to them at 0.002 ppm at 17° C. in 24 hours. Channel catfish were the least sensitive but they succumbed to 0.8 ppm of aldrin which was the least toxic of the six insecticides.

Rotenoids:--The tests begun in 1962 on commercially-available preparations of rotenone were continued and expanded to include additional agents and fishes. It is apparent that the varying quantities of active ingredients in the formulations make accurate comparisons of potency very difficult. It was observed also that most of the fourteen species of fish varied in their degree of response to each formulation and there was no consistent pattern among the responses to the ten formulations.

One series of tests indicated that there was no significant difference in the toxicity of rotenone to sac-fry and large fingerlings of rainbow trout and brook trout. Other tests with synergized formulations gave equal or better performance on most fishes but failed to show up as well as straight, high-rotenone preparations against such tolerant species as goldfish and black bullhead.

An opportunity was presented in May to attempt a multiple control of fish, fish disease, and aquatic weeds at the Wolf Springs National School of Forestry in northern Wisconsin. The problems were centered in three tandem trout ponds and involved: yearling and older brook trout which were heavily infested with copepods (*Salmincola edwardsii*) and tapeworms; abundant mudminnow, cyprinids, stickleback, and sculpin; and algae and higher plants. Most of the trout were removed with electrofishing gear and an unsuccessful attempt was made to kill their parasitic copepods with malathion. Temporary control of the aquatic plants was achieved by drawdown and by applications of herbicides.

A commercial preparation of rotenone was employed to exterminate the remaining trout in the ponds and to drastically reduce the number of other fishes. A 1-ppm concentration was applied for 8 hours at two head springs of the upper pond, and it was important that no rotenone escape from the third pond into the outlet trout stream. Detoxification of the chemical in the 1,100-gpm outlet discharge was accomplished well by means of potassium permanganate at 1 ppm from drip stations and a barrier composed of sacks of activated charcoal. Hourly analyses for rotenone in the pond and in the discharge were performed. Toxic levels were achieved in all ponds, and complete detoxification was accomplished in the discharge. No traces of chemical were found in the ponds after the 20 hours.

Antibiotic:--Major efforts were devoted to a fungicidal antibiotic which exhibits fine potential as a fish toxicant. Its outstanding properties include: 1) toxic to fish at exceedingly low concentrations; 2) toxic to carp, suckers, sunfish, and perch at concentrations which permit other species to survive; 3) relatively harmless to fish food organisms; 4) relatively

non-toxic to amphibians, birds, and mammals; 5) apparently harmless to plant life; and 6) degrades rapidly without detectable residue.

The antibiotic was tested in the laboratory against 25 species of fish, fish food organisms, and aquatic vertebrates. The fish were killed within a concentration range of 0.1 to 120 ppb. Trials were made also of the irreversible mode of action of the toxicant, of its shelf life in aqueous and acetone solutions, of its effectiveness against fish in two formulations, and of its degradation in waters of various pH levels. A manuscript dealing with the results of these studies is in preparation.

The antibiotic was given preliminary trials in the field with cooperation from the Wisconsin Alumni Research Foundation, S. B. Penick Company, and the Wisconsin Conservation Department. Crystalline grade and an experimental formulation were applied at 10 ppb in two ponds, 0.47-acre and 0.78-acre, at the Delafield Warmwater Fisheries Research Station. The smaller pond had been stocked with 16 species at 240 pounds per acre, and the larger pond with 17 species at 225 pounds per acre. The results are presented in Tables 1 and 2.

The formulated material caused fish to begin coming to the surface in 4 to 6 hours after application whereas response to the acetone solution of crystalline material did not begin until 14 to 16 hours. There was, however, no discernible difference between the two in overall effectiveness at 24 hours. Northern pike were the first to show distress followed by rainbow trout, white sucker, carp, walleye, and sunfish. The majority of fish were dead and removed within the first 24 hours. Longnose gar, bowfin, and bullheads were not affected at the low concentration.

There was rapid degradation of the antibiotic within 72 hours after application, and sensitive species placed in bioassay cages after that time showed no ill effects. Observations continued for 20 days and the ponds were then drained to recover the surviving gar, bowfin, and bullheads. Nearly 1,000 black bullheads

were transported to La Crosse to check continuing survival. There was no significant mortality within two months.

Blood samples were taken from 20 of the surviving black bullheads and analyzed. The data are summarized in Table 3.

Comparisons were made on the kinds and numbers of bottom organisms present in the ponds before and after treatment. In general, there were no significant effects attributable to the toxicant.

Two series of quasi-field tests of the antibiotic were made in plastic pools in July and October. Differences in biological activity were detected in pools with sand or loam bottoms. Alkalinity is a primary factor in the rate and extent of degradation and the antibiotic is short lived in waters with high pH. Furthermore, there was no great harm to plankton, bottom fauna, or aquatic plants in these tests.

Cresylic acid isomers:--The ortho-, meta-, and para- isomers of cresylic acid were bioassayed with 12 species at three temperatures. Fish demonstrate erratic responses to cresols and difficulty is encountered in establishing concentrations which produce all-or-none effects. Also, the time required to anesthetize or kill fish is variable within a species. In contrast to most compounds which are active against fish, increasing water temperatures require higher concentrations of cresols to cause responses. For example, northern pike were killed by 6 ppm of para- isomer at 12° C. in 24 hours but 20 ppm was necessary at 22°.

The para- was the more toxic of the isomers tested. Of the fish, catfishes exhibited the greater resistance and concentrations as high as 50 ppm were needed to kill.

Endothal derivatives:--Compounds of this class include known herbicides, bactericides, and fungicides. The results of advanced tests on two indicated both piscicidal and herbicidal activities and interest in them continues because of their potential as dual control agents.

Table 1:--Mortality of selected fish caused by 10 ppb of formulated fungicidal antibiotic in a 0.47-acre pond.

Fish	Number stocked	Average length (inches)	Average weight (pounds)	Percent mortality at			
				24 hours	48 hours	96 hours	20 days
Longnose gar	3	25.6	1.45	0	0	0	0
Bowfin	1	16.8	1.20	0	0	0	0
Rainbow trout	312	4.0	0.18	100	...	...	...
Northern pike	7	17.8	1.57	72	72	72	100
Goldfish	740	2.4	0.02	100	...	...	...
Carp	18	15.3	2.48	94	100	...	...
White sucker	4	15.1	1.22	75	75	75	100
Black bullhead	600	3.8	0.04	0	0	0	0
Yellow bullhead	4	8.3	0.37	0	0	0	0
Brown bullhead	1	4.2	0.11	0	0	0	0
Rock bass	1	8.0	0.30	100	...	...	...
Green sunfish	3	3.8	0.03	0	0	33	100
Pumpkinseed	13	4.6	0.09	85	92	100	...
Bluegill	27	6.1	0.15	78	78	82	100
Sunfish (hybrid)	1400	1.7	0.02	100	...	...	...
Black crappie	7	8.3	0.21	72	72	72	100
Largemouth bass	4	15.4	1.75	25	100	...	...
Walleye	1	13.5	0.70	100	...	...	...

Table 2:--Mortality of selected fish caused by 10 ppb of crystalline fungicidal antibiotic in a 0.78-acre pond.

Fish	Number stocked	Average length (inches)	Average weight (pounds)	Percent mortality at			
				24 hours	48 hours	96 hours	20 days
Longnose gar	3	24.6	0.87	0	0	67	67
Bowfin	1	21.8	3.90	0	0	0	0
Rainbow trout	470	4.1	0.19	100	...	...	...
Northern pike	8	19.1	2.15	80	80	100	...
Goldfish	1400	2.7	0.02	100	...	...	...
Carp	27	15.3	2.45	96	100	...	...
White sucker	6	15.7	1.40	80	80	80	100
Black bullhead	875	3.7	0.03	0	0.2	0.3	18
Yellow bullhead	1	5.7	0.13	0	0	0	0
Brown bullhead	6	11.4	0.83	0	0	0	20
Rock bass	1	8.2	0.30	0	0	0	100
Green sunfish	4	4.2	0.05	0	25	50	100
Pumpkinseed	24	4.6	0.07	8	50	50	100
Bluegill	43	6.4	0.19	44	65	84	100
Sunfish (hybrid)	2055	1.7	0.02	100	...	...	...
Black crappie	9	8.8	0.30	11	44	44	100
Largemouth bass	5	12.8	1.05	0	40	80	100
Walleye	2	13.0	0.85	100	...	...	...
Freshwater drum	1	11.6	0.60	0	0	100	...

Table 3:--Some hematological measurements on the blood of 20 black bull-  
heads which survived exposure to a fungicidal antibiotic.

Measurement	Individual samples		Pooled samples
	Mean	Range	
Hematocrit	36.6 %	32.0-54.0 %	-
Hemoglobin	11.0 gm. %	8.5-14.0 gm. %	-
RBC count	1.97 million/mm <sup>3</sup>	1.28-2.46 million/mm <sup>3</sup>	-
pH	-	-	7.42
Carbon dioxide	-	-	20.98 mEq/liter (46.77 Vol. %)
HCO <sub>3</sub> <sup>-</sup>	-	-	20.01 mEq/liter
H <sub>2</sub> CO <sub>3</sub>	-	-	0.97 mEq/liter
Ratio	-	-	20.6:1
Total protein	-	-	3.11 gm. %
Albumin	-	-	1.48 gm. %
Globulin	-	-	1.63 gm. %
A/G ratio	-	-	0.91
Creatinine	-	-	0.7 mg. %
Bilirubin	-	-	none indicated

Table 4:--Hematocrit values obtained with electronic and microhematocrit apparatus  
from single specimens of seven species of fish.

Species	Hematocrit								Differ- ence between means
	Electronic				Microhematocrit				
	Number of repli- cates	Mean	Range	Stand- ard devi- ation	Number of repli- cates	Mean	Range	Stand- ard devi- ation	
Shovelnose sturgeon	12	15.5	14.0-17.0	0.8	12	22.0	20.5-23.0	1.0	6.5
Spotted sucker	12	20.0	17.0-23.0	1.4	10	33.5	32.0-34.0	0.7	13.5
Northern redhorse	20	31.0	28.5-32.5	1.2	24	33.0	31.0-34.5	0.7	2.0
Brown bullhead	12	16.0	14.0-18.0	0.9	12	22.0	21.0-22.5	0.5	6.0
White bass	12	14.0	12.0-18.0	1.5	11	31.0	30.0-32.5	0.7	17.0
Large- mouth bass	8	14.5	12.0-16.0	1.4	12	26.0	24.0-27.0	1.0	11.5
Walleye	9	28.0	25.0-29.0	1.4	12	40.0	39.0-41.5	0.7	12.0



Miscellaneous:--This group includes compounds which cannot be classified otherwise because structures or properties are unknown. Several were very toxic to fish at low concentrations. Also, 45 experimental mixtures of 9 compounds were bioassayed. The results indicated that there was synergism in 17 mixtures and increases in the range of selective toxicity in 10 mixtures.

## PHYSIOLOGY

### Hematocrits in fish

The YSI, Model 30, Electronic Hematocrit was evaluated for use in fisheries. Measurements obtained with this instrument and with the microhematocrit technique on blood from seven species of fish were compared (table 4). The electronic hematocrits were consistently low. Moreover, the instrument showed that with continuous reading of a given sample the percentage gradually increased in possible correlation with the sedimentation of erythrocytes.

Other investigators have reported that the electronic hematocrit of human blood increases with higher levels of blood protein. Also, the hematocrits obtained by electronic and micro- techniques are approximately the same at a protein concentration of 7.4 grams percent. A parallel relationship was observed with blood from longnose gar and rainbow trout, but the hematocrit values coincided at a protein concentration of 14 grams percent. The difference may be related to the sizes of erythrocytes in humans and fish.

The protein concentrations of the fish blood were adjusted to a range of 1.5 to 15 grams percent by additions of bovine albumin. The electronic hematocrits averaged 17 percent less than microhematocrits at 1.5 grams protein and 2 percent more at 15 grams protein. There was little difference between the two species of fish.

It was concluded that the disadvantages of the electronic hematocrit instrument outweigh the advantages. Although it requires only 0.02 cc of blood, gives instantaneous

readings, and is easily portable, the variable and marked deviations from the microhematocrit compromise its use in fish work. Also, large numbers of samples cannot be processed at a time as with the micro- technique.

### Chemical residues in fish

Thiodan is a chlorinated hydrocarbon which shows promise in fish control. It is comparable to endrin in toxicity to fish and appears to be degradable under alkaline conditions. Methods for determining its residues in fish flesh have been under investigation, including paper chromatography, colorimetric analyses, and a radio-active tracer.

Paper chromatography was used to separate and identify standards of thiodan and thiodan alcohol. Applications of 2.5 to 5.0 mmg were detectable. Experiments with C-14-labeled thiodan were initiated by chromatography showed that the material had decomposed into what seemed to be thiodan alcohol. There were also problems in the clean-up of tissue extracts. Fatty material persisted and further clean-up with fuming sulfuric acid destroyed the toxicant. In several trials, thiodan at 0.1 and 0.01 ppm killed carp and largemouth bass, but the toxicant could not be detected on chromatograms of extracts from 25 to 50 grams of tissue.

A relatively new colorimetric analysis for thiodan is promising. Acetonitrile and a Florisil-carbon column are used for clean-up and a recovery of approximately 70 percent of thiodan was achieved. The analysis is sensitive to 5 mmg, relatively specific to thiodan, but thiodan alcohol also reacts. Two goldfish which were exposed daily to fresh solutions of thiodan at 0.0075 ppm died after 10 days. The subsequent colorimetric analysis showed approximately 2 ppm of thiodan in the fish tissues.

## OTHER

### Electrofishing

Substantial improvements were made in shocker gear for the small and large boats, and a hand-operated system was constructed. Successful applications were made in the Mississippi

and softwater trout ponds. Also, assistance and gear were provided to the Branch of Fishery Management Services during trout surveys on Camp McCoy Military Reservation.

### Aquarium

Attractive innovations were made in the public aquarium and largely through the assistance of the Branch of Fish Hatcheries. It was opened in early May to accommodate the large numbers of school groups on tour and was closed for the season on October 1.

### Correspondence courses

Fourteen members of the La Crosse and Warm Springs staffs received approval to take a total of 16 courses in Technical Writing, Statistical Methods in Biology and Agriculture, Statistics of Biological Assay, and Experimental Design from the Department of Agriculture Graduate School.

### Developments at Warm Springs

A major portion of the constructing, equipping, and staffing of facilities was completed during the year. The laboratory building was accepted and activated in July. A staff of three fishery biologists, two chemists, and a management assistant was placed on duty. A \$11,000 contract for filling a pond and grading

a site for future construction was accomplished. The erection of a wet-lab-holding house and a storage building under a \$78,555 contract was nearly completed. Preliminary plans for outdoor pools, an auxiliary water supply, bank stabilization, grading, and paving were prepared.

Five members of the staff spent 2 to 6 months in training at La Crosse. Upon activation of the laboratory, they proceeded to set up a library, to furnish and equip laboratories and offices, to investigate sources of experimental fish from hatcheries and natural waters, to acquire and catalog a reference collection of fish, and to conduct experiments in plastic pools on the grounds of the Warm Springs National Fish Hatchery.

Approximately three dozen plastic pools, 9 and 10 feet in diameter, were employed in experiments to find a practical indicator of the gross or general effects of fish toxicants on entire aquatic communities. The pools contain bottom soils, plants, plankton, and other aquatic organisms. Measurements of community metabolism are made by two methods before and after treatment with fish toxicants to detect and evaluate immediate and persistent effects, if any, on the ecosystems. These observations and others to assess the practicality of plastic pools for all-season bioassays are continuing into 1964.

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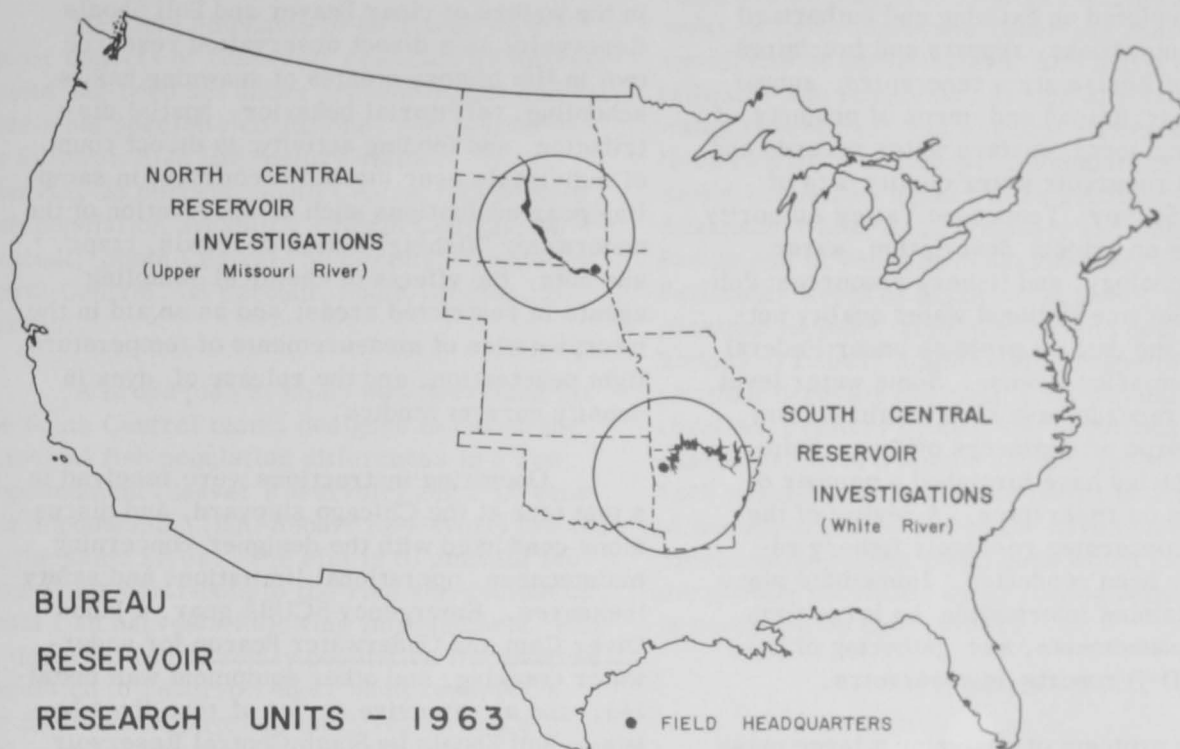
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# RESERVOIRS

## NATIONAL RESERVOIR RESEARCH PROGRAM

Fayetteville, Arkansas

Robert M. Jenkins, Director



### HIGHLIGHTS

Search and procurement of available biological, physical and chemical data concerning large reservoirs began in July. Pertinent materials have been obtained from the Branch of River Basin Studies, Bureau of Sport Fisheries and Wildlife, Geological Survey, Bureau of Reclamation, Corps of Engineers, Tennessee Valley Authority, Public Health Service, and Federal Power Commission. Cataloguing of raw data and development of methods of storage and retrieval are proceeding, with the goal of identifying hitherto unrecognized interrelations between reservoir environmental conditions, biological productivity, and sport fish production and harvest.

Initial testing of a two-man, dry submarine was conducted in Bull Shoals Reservoir in October. Results were generally satisfactory. Water clarity and maintenance requirements

appear to be the controlling factors restricting the use of this new research tool.

Broad plans were developed for expansion of the program of reservoir research nationally to solve pressing problems concerning the fishery resources of large Federal reservoirs and contiguous streams.

### RESERVOIR DATA COLLECTION

In an attempt to collate and analyze existing information concerning the physical, chemical and biological characteristics of U.S. Reservoirs (over 500 surface acres at "normal" level), published and unpublished materials are being assembled from all known sources. The aim is to correlate pronounced fish production differences with such variables as drainage basin geology, soils and vegetation, climatology, reservoir age and use(s), shoreline configuration, water level fluctuation, inflowing water quality, water exchange

rate, discharge levels, plankton, bottom fauna, and other limnological features, and preimpoundment fish populations.

The following information has been obtained and is being catalogued by individual reservoir: All Branch of River Basin Studies reports completed on existing and authorized impoundments; books, reports and brochures on Bureau of Reclamation reservoirs; annual reports, descriptions and maps of projects of Corps of Engineers; surface water records and stream and reservoir water quality data of Geological Survey; Tennessee Valley Authority publications on project description, water quality, limnology, and fishery resources; Public Health Service National water quality network data; and data on projects under Federal Power Commission license. Some water level fluctuation records have been obtained from TVA and Corps of Engineers offices. State fishery agencies have furnished a number of publications on reservoirs. A review of the literature concerning reservoir fishery resources has been conducted. Immediate plans call for obtaining information on large non-federal impoundments, and gathering of unpublished (D-J) reports on reservoirs.

The problem of analyzing a large mass of descriptive data concerning approximately 1,000 reservoirs will involve 1) cataloguing information by specific trait or category on individual reservoir data sheets; 2) storage of data on punch cards for preliminary analysis; and 3) transfer to IBM cards or tape for final computer correlation studies. Success in identifying environmental factors (or groups of factors) which influence fish production will depend in large measure on the existence of biological findings which can be accurately interpreted for use in comparative analyses of statistical significance.

#### SUBMARINE AS RESEARCH TOOL

A two-man, dry submarine built by the American Submarine Company, (101st Street and Calumet River, Chicago) was procured in August. This battery-powered vehicle has an overall length of 13 feet, a 50-inch beam, and weighs 1 ton. Top speed is 6 knots, with 2

speeds ahead and astern, a cruising range of 15 miles, and a payload of 520 pounds. Each occupant has 360-degree visibility through a cylindrical plexiglass section in each conning tower, and viewports overhead and in the bottom.

It is planned to evaluate the submarine in the waters of clear Beaver and Bull Shoals Reservoirs as a direct observation research tool in life history studies of spawning habits, schooling, territorial behavior, spatial distribution, and feeding activity; in direct count of individuals over measured courses; in sampling gear evaluations such as observation of the underwater "fishing" action of trawls, traps, and nets, the effects of chemical sampling agents in restricted areas; and as an aid in the interpretation of measurements of temperature, light penetration, and the release of dyes in density current studies.

Operating instructions were received in a test tank at the Chicago shipyard, and discussions conducted with the designer concerning maintenance, operational limitations and safety measures. Emergency SCUBA gear, a Janus Diver Com and Underwater Beacon for underwater tracking, and other equipment was installed, and an extensive series of test dives begun in Bull Shoals by South Central Reservoir Investigation biologists. Testing was terminated upon breakdown of a compressor motor coupling, and the onset of cold weather. From the initial test dive experience, it was evident that the major limitations of the submarine as a research vehicle are imposed by water clarity and maintenance requirements. Further testing and full-scale field operations will be resumed in the spring (minimum water temperature, 60° F.).

#### RESERVOIR RESEARCH TEAM COORDINATION

To provide optimum coordination of effort between the Reservoir Investigation teams, an interstaff meeting was held at the Yankton, South Dakota field headquarters in November. Specific problem approaches, methodology, data collecting and recording systems, and innovations in limnological and fish sampling equipment were discussed by team members. Particular emphasis was placed on early life history and food

habits studies, underwater observations, fish tagging, trawls and trap nets, electrofishing gear, the automatic plankton sampler and water quality monitor, fluorescent dye use in current measurement, and university contract research accomplishments.

Relative emphasis to be placed on the major aspects of reservoir research by the two teams has been set under present budget and personnel specialty conditions, approximately as follows: Fish life history studies - North Central, 25 percent; South Central, 30 percent; fish population dynamics - North Central, 40 percent; South Central, 50 percent; limnology North Central, 35 percent, South Central, 20 percent.

A broad plan of study was developed for the South Central team, designed to define the principal fish population differences in a new impoundment (Beaver Reservoir) and a 12-year-old impoundment (Bull Shoals Reservoir) located on the same river. The aim is to pinpoint factors which contribute to the rise and decline of sport fish harvests following impoundment, and to describe concurrently population fluctuations unrelated to reservoir age. Both reservoirs lie in the same physiographic region and are subjected to similar climatic influences. Two reservoirs lie between them on the White River, reducing the direct influence of the upstream one on the other. By employing a single team of biologists, a high degree of standardization may be obtained of intensive observation, data collection, gear manipulation, total effort, and data analysis. This will continue through the first 6 to 10 year "new" impoundment cycle.

## PLANNING FUTURE RESERVOIR STUDIES

A broad plan to guide the implementation of additional reservoir research in major river basin areas has been prepared. The principal fishery research problem areas posed by reservoirs are embodied in 1) large, warmwater, multi-purpose interstate reservoir chains, 2) the effects of multiple-level dam outlet on the tailwater and reservoir, 3) high altitude cold-water reservoirs, and 4) small warmwater flood control reservoirs. Immediate needs and unmet challenges appear to be greatest in the Arkansas-White-Red, Missouri and Ohio River Basins and in Western mountain reservoirs. Particular fields of inquiry that should be emphasized include: reproduction and early life history of fishes; inter- and intraspecific competition; behavior patterns; total population estimation; primary production; physicochemical characteristics; plankton and bottom fauna crops. Such research will lead to a better understanding of manmade lakes, and to the development of improved management techniques which can satisfy the greatly increased sport fishing demand that is predicted on these waters.

### STAFF

Mr. Robert M. Jenkins, Fishery Biologist



# NORTH CENTRAL RESERVOIR INVESTIGATIONS

Yankton, South Dakota  
Norman G. Benson, Chief

## HIGHLIGHTS

Studies of Lewis and Clark Lake since impoundment in 1956 by South Dakota Department of Game, Fish, and Parks (1956-1961) and Bureau of Sport Fisheries and Wildlife (1962-1963) have revealed that the abundance of most fishes has decreased and spawning success has been poor for most species since 1957. In contrast, increasing numbers of sauger, freshwater drum, and white bass (planted in 1959) have been recorded.

Most of the spawning activity of the sauger population appears to occur in the Fort Randall tailwater, 40 miles upstream from the upper end of Lewis and Clark Lake. Abundance of this species in the reservoir has apparently quadrupled since 1956.

Density of most zooplankters in Lewis and Clark Lake in 1963 showed an increase with depth and was greater in the lower end than in the upper portion of the reservoir. A fine layer of silt covers much of the bottom, characterized by low production of bottom organisms. A hard clay-rubble bottom covers most of the shoreline areas which in contrast produces large numbers of organisms.

A water quality monitoring system was installed in the Gavins Point powerhouse to measure automatically dissolved oxygen, pH, conductivity, turbidity, and temperature. An automatic plankton sampling system also was designed, built, and installed in the powerhouse for quantitative collection and preservation of outflowing plankton.

The electroshocking equipment was improved by incorporation of a variable power transformer. Analysis of catch-effort records showed that evening (5-9 PM) sampling is the most efficient period for fish collection.

Plans were developed for establishing and staffing a research station on Oahe Reservoir.

## EVALUATION OF FISH SAMPLING TECHNIQUES

Fish sampling in Lewis and Clark Lake, a main-stem Missouri River reservoir, was continued in 1963 with improvement in methods as a result of previous years' study. Sampling gears used were 350-foot gill nets, frame nets, 16 and 28-foot semi-balloon trawls, and an electric shocker. Egg, fry, and plankton nets (modified Miller sampler) were fished in the limnetic zone to determine spawning success. Excluding the catch in the limnetic zone, approximately 39,000 fish, comprising 35 species, were collected in 1963. Increases in the relative abundance of drum and white bass were noted (table 1).

Analysis of data collected suggests that a combination of fishing gears is necessary to sample the fish population adequately. Each gear sampled a different segment of the fish population. Available evidence indicates that the presently employed gears sampled most ages and sizes of fish in the reservoir. Further modification of existing gear, and new sampling methods have been planned.

### Net fishing

Fish collections obtained by gill and frame nets in 1963 were similar to 1962. Trawls fished

Table 1:--Percent distribution of major fish species collected in Lewis and Clark Lake, 1962-63 (not including gizzard shad or minnows).

Species	1962	1963
Carp	32	29
River carpsucker	23	20
White crappie	7	6
Sauger	7	6
Freshwater drum	5	9
Channel catfish	5	4
Smallmouth buffalo	4	4
White bass	2	6
Other	15	16

in 1963 contained a 1/4-inch bar-mesh liner in the cod end to improve catch of juvenile fishes. A 28-foot trawl, fished in waters 10 to 40 feet deep, was several times more effective than the 16-foot trawl in capture of young-of-the-year and juvenile fishes. Collections made with the 16-foot trawl, fished in waters 3 to 10 feet deep, were similar to those made with the electric shocker. On the basis of these findings, the 16-foot trawl will be discontinued as a regular sampling method.

Egg, fry, and plankton nets were adopted as regular sampling methods in 1963. Fished from the surface to a depth of 20 feet they were effective in collecting fish less than 30 mm. long. Future work with these fine mesh nets will include sampling of bottom waters.

#### Electrical fishing

The relation of time of day to shocker catches was studied. Criteria for evaluation were total catch, number of species, and size range. During the summer months the weekly shocker schedule was alternated between morning (9:00 a.m. to 12:00 p.m.) and evening (5:00 p.m. to 9:00 p.m.) sampling. Results indicated that evening sampling was 70 percent more efficient for most species (table 2). Size range and species collected were similar. Supplementary studies were conducted twice during the summer in two different locations over a 24-hour period. Peak catches were obtained in the evening (5:00 p.m. to 9:00 p.m.). Night sampling (1:00 a.m. to 5:00 a.m.) produced the poorest catches. Size range and species collected were similar among sampling periods. This being the case, electric shocking for monitoring purposes will henceforth be conducted during evening hours.

To standardize the operation and efficiency of the electric shocker, a variable power transformer was installed in August, 1963. With this unit a relatively constant amperage can be maintained regardless of changes in water resistivity. At present, shocker sampling is conducted between 2.5 and 3.5 amperes. Water resistivity in Lewis and Clark Lake ranges between 1,100 and 1,200 ohms.

Analysis of fish collection data obtained by the South Dakota Department of Game, Fish, and Parks, 1956-1961, and the Bureau of Sport Fisheries and Wildlife in 1962 and 1963, indicates that the fish population in Lewis and Clark Lake is dominated by carp and river carpsucker, with relatively few sport species. Total numbers of fish have apparently decreased since 1957. The decrease in sport fish has been greater than rough fish.

The fish population has not stabilized since impoundment. The 1956 year class, strong for most species, has dominated the population to date. Reproduction and growth were good for all fishes during the first year of impoundment, but have been poor for most species since. Reasons for poor reproduction and growth are unknown. They may be related to the pattern of reservoir water level fluctuation as previously suggested by South Dakota Department of Game, Fish, and Parks biologists, or to other less obvious environmental influents.

#### Relative abundance

Numbers of fish captured by gill and frame nets in June, July, and August, 1956 through 1963, were used to determine trends in abundance of species vulnerable to these gears. Most abundant species were carp, river carpsucker, and white crappie. Other major species listed phylogenetically, were shovelnose sturgeon, shortnose gar, smallmouth buffalo, channel catfish, black crappie, sauger, and freshwater drum.

Considering gear selectivity, effort by year, and equating the differences in catch between gill and frame nets, it was possible to develop relative abundance estimates from 1956 to 1963 (Table 3). The base year is 1956 which has a relative abundance index of 1.

Apparent abundance of most fishes declined between 1956 and 1963. Marked decreases were suggested for shovelnose sturgeon, carp, and black crappie. Both white and black crappie experienced wide fluctuations in abundance and

in 1963 were at a relatively low level. Black crappie was predominant in 1957 but between 1958 and 1962 declined to a minor role compared to the white crappie. The abundance of short-nose gar, smallmouth buffalo, and river carpsucker has remained rather stable since 1957. Sauger abundance has increased since the early years of impoundment and freshwater drum abundance increased in 1962 and 1963 compared to that of previous years.

Shoreline seining has been conducted since 1956 to determine relative abundance of small fishes. The silvery minnow, (Hybognathus nuchalis), which was abundant following formation of the reservoir, declined to a low level and the emerald shiner (Notropis atherinoides) became the dominant cyprinid species. Gizzard shad, relatively rare prior to impoundment, became the most abundant forage species.

#### Fish marking

Studies were conducted during the year to determine if nylon streamer and Petersen disk tags were suitable for marking carp, river carpsucker, channel catfish, and sauger. For each species, 100 were tagged with streamer tags, 100 with Petersen tags, and 100 were used as controls. After handling, all fish were dipped in 70 ppm. malachite green and placed in ponds. Tagging was completed in October, 1962 and all fish were held until March, 1963 when ponds were drained. Fish were fed standard dry food and minnows.

Survival of carp and river carpsucker tagged with the streamer tag was good. Channel catfish survival was poor with both types of tag, but the control lot had no mortality. The catfish study will be repeated since the tagged and the control lot were obtained from different sources. Survival of tagged sauger was fair. For both types of tags, mortality decreased with increase in fish length. Sauger survival was higher in those fish marked with the streamer tag.

#### Reservoir tagging studies

In the spring of 1963, 855 sauger and walleye were tagged with a nylon streamer tag and

Table 2:--Average catch-per-unit effort of major fish species by electric shocker, June-August, 1963.

Species	Morning		Evening	
	No.	Size range (mm.)	No.	Size range (mm.)
Gizzard shad	143	25-145	274	35-155
Carp	15	120-565	25	50-545
River carpsucker	7	55-340	13	45-360
Smallmouth buffalo	2	295-380	4	285-375
White bass	5	40-175	2	45-275
Sauger	3	50-145	6	70-585
Freshwater drum	3	60-350	9	45-370
Average C/E exclusive of shad	35		59	

Table 3:--Relative abundance of major fish species compared to 1956, Lewis and Clark Lake, 1956-63.

Species	1956	1957	1958	1959	1960	1961	1962	1963
Shovelnose sturgeon	1.0	1.0	0.1	0.2	0.1	0.2	0.3	0.1
Shortnose gar	1.0	1.3	1.1	1.0	1.4	1.6	0.4	0.6
Carp	1.0	0.7	0.2	0.2	0.1	0.2	0.1	0.1
River carpsucker	1.0	1.0	0.8	0.6	0.7	0.8	0.8	0.6
Smallmouth buffalo	1.0	14.0	13.0	10.0	12.0	7.0	9.0	10.0
Channel catfish	1.0	0.5	0.3	0.3	0.3	0.4	0.5	0.3
White crappie	1.0	2.4	11.2	7.1	1.5	3.8	1.5	1.4
Black crappie	1.0	38.1	18.2	6.7	1.0	0.7	0.3	0.1
Sauger	1.0	2.4	2.9	4.2	5.1	3.7	3.9	3.9
Freshwater drum	1.0	0.5	0.6	0.4	0.5	0.7	3.5	2.0

released in the tailwaters of Fort Randall Dam to determine movement between the tailwater and Lewis and Clark Lake. By the end of 1963, 66 tags (7.7 percent) were returned by anglers. Most recoveries were from the vicinity of tagging, but three (sauger tags) were returned from the mouth of the Niobrara River, 40 miles downstream.

During the fall of 1963 an additional 402 fish were released in the following locations:

	Sauger	Walleye	Total
Lake	156	8	164
River	79	2	81
Tailwater	94	63	157
	329	73	402

To date, 10 of these tags have been returned; 5 from the tailwater, 3 from the river, and 2 from the lake. All recoveries were from location of release.

### Year class abundance

Aging of major fish species collected in 1963 has corroborated both year class abundance as determined from previous years collections and 1962 reproduction measurements (table 4). Both carp and carp sucker have had poor reproduction since 1957. Channel catfish reproduction has been erratic since 1956 with good survival in 1960 and 1962. White bass, first introduced into the reservoir in 1959, produced a strong year-class in 1962. Reproductive success of white crappie in 1962 was the highest since 1958. Both sauger and drum produced strong year-classes in 1962.

### LIFE HISTORIES OF RESERVOIR FISHES

Life history studies in 1963 emphasized the very early stages since it appears that they represent the most critical period for survival in reservoirs. In addition, food habits of selected non-predaceous species were determined.

### White crappie

Studies on the early life history of the white crappie for determination of factors controlling year-class abundance were continued for the second summer. Location and observation of spawning could not be accomplished because of turbid water conditions.

Intensive sampling, especially in mouths of creeks and backwaters, resulted in capture of 3,954 young-of-the-year. Drawings of white crappie fry ranging in length from 5.5 to 13.0 mm. were made for identification references. The following juvenile characteristics were determined by sampling area; seasonal growth, length of spawning time and period of peak spawning, and length-weight relationship. Food habits were determined from examination of 208 young-of-the-year stomachs.

The body-scale relationship was calculated from key scales taken from age I and older

fish collected in 1963. Length at scale formation and end of first year of growth was also calculated. Length-weight relationship and growth characteristics for fish older than young-of-the-year were determined.

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Life history studies were complicated by the fact that both sauger and walleye spawn in the tailwaters at approximately the same time. Estimated time of both sauger and walleye spawning was May 1-15, when the water temperature ranged from 48 to 54° F. We were unable to capture ripe sauger; spent fish were collected. Both eggs and newly hatched fry were collected below the study area in fry nets set after May 21. We have been unable to distinguish walleye from sauger fry at lengths less than 40 mm. Walleye reportedly have a 2-week pelagic existence during the egg-sac fry stage and we suspect a similar life history for the sauger.

Table 4:--Percent age-class distribution of major fish species collected in Lewis and Clark Lake, 1963.

Age	Year Class	Species						
		Carp	River carp sucker	Channel catfish	White bass	White crappie	Sauger	Drum
I	1962	7	4	20	96	96	50	66
II	1961	2	4	3	4	4	22	5
III	1960	-	1	14	2	3	12	6
IV	1959	2	3	9	-	4	7	6
V	1958	4	10	4	-	21	4	9
VI	1957	15	45	8	-	14	3	7
VII	1956	35	27	23	-	29	2	3
1								
VIII	1955	21	4	14	-	7	-	-
IX	1954	10	1	3	-	1	-	-
X*	1953	4	1	2	-	-	-	-
Total		100	100	100	100	100	100	100

1/ Reservoir formed July, 1955



in 1963 were at a relatively low level. Black crappie was predominant in 1957 but between 1958 and 1962 declined to a minor role compared to the white crappie. The abundance of short-nose gar, smallmouth buffalo, and river carp-sucker has remained rather stable since 1957. Sauger abundance has increased since the early years of impoundment and freshwater drum abundance increased in 1962 and 1963 compared to that of previous years.

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II	1961	2	4	3	4	4	22	5
III	1960	-	1	14	2	3	12	6
IV	1959	2	3	9	-	4	7	6
V	1958	4	10	4	-	21	4	9
VI	1957	15	15	8	-	14	3	7
VII	1956	35	27	23	-	20	2	3
VIII	1955	21	4	14	-	7	-	-
IX	1954	10	1	3	-	1	-	-
X*	1953	4	1	2	-	-	-	-
Total		100	100	100	100	100	100	100

\*/ Reservoir formed July, 1955

## Food habits

A contract was established with South Dakota State College to analyze stomach contents of the following non-predaceous fishes; gizzard shad, carp, river carpsucker, bigmouth buffalo, smallmouth buffalo, and northern redhorse. Samples were collected between May and October and include both juvenile and adult fish. Phytoplankton was the dominant food for gizzard shad, carp, river carpsucker, and smallmouth buffalo. Zooplankton was the dominant food for bigmouth buffalo; higher plant material for northern redhorse. Except for northern redhorse and bigmouth buffalo, aquatic insects were scarce in the stomachs examined. Detritus was common in all stomachs. The relationship of this information to growth rates, mortality, and food abundance will be studied.

## LIMNOLOGY

### Zooplankton

Drift and tow net samples:--Plankton samples were collected during the summer of 1962 with a Miller sampler. Samples were taken at 5 foot intervals from the surface to a depth of 30 feet. All zooplankters were identified to genus and distributions were plotted according to depth, time, and transect position in the reservoir.

Maximum densities of all organisms, except Diaptomus, were observed in the lower section of the reservoir. Diaptomus was abundant both in river drift samples and in the upper section of the reservoir. Densities of Diaptomus and Cyclops generally increased with depth with maximum concentrations near the bottom. Other genera showed maximum concentrations at depths between 5 and 30 feet.

During the 1963 sampling season, 176 zooplankton samples were collected in the reservoir with metered Miller samplers. These samples were taken from limnetic areas, littoral areas, tributary streams, and boat basins; both vertical and horizontal tows were used.

Analyses of these samples for species composition indicated 22 genera and 40 species. The majority of these species were encountered infrequently, and only 8-10 species were abundant at any given sampling date.

Powerhouse plankton sampling:--A sampling unit containing three stainless steel screens of #00, #10, and #20 size, was installed in the powerhouse of Gavins Point Dam during January to sample plankton passing through the dam. The following problems were encountered: (1) algae clogged the screens resulting in sample loss; (2) organisms frequently passed through the #20 screen; and (3) spoilage prevented long term use.

Therefore, a new unit was designed and installed in November (figure 1). This unit automatically preserves the samples, shuts off when the screens clog, and takes 250 mm. samples of the screened water. The unit samples one out of every six hours on a weekly basis, and sample concentrates contain organisms filtered from approximately 4,000 gallons of water.



Figure 1:--Automatic plankton sampler

## Primary production

An investigation of primary productivity is being conducted using the  $C^{14}$  and  $O_2$  light-and-dark bottle methods. Qualitative and quantitative studies of phytoplankton populations are being conducted simultaneously.

## Bottom organisms

A total of 218 bottom samples were collected in Lewis and Clark Lake during 1963. In general, the lake bottom is covered by a layer of silt except in shallow areas influenced by wave action and in the upper river channel. This bottom type is dominated by Hexagenia, Chironomidae, and Oligochaeta, and the abundance of these organisms appears to vary inversely with the extent of siltation.

The three distinct bottom types occurring along the margin of the shore are hard clay rubble, sand, and eroded soil. The hard clay rubble type, which constitutes approximately 90 percent of the shore area, is inhabited by large populations of Chironomidae, Baetidae and Trichoptera. The sand areas contribute little biomass, and soil areas produce, almost exclusively, Chironomidae.

Three shore areas were sampled during July to determine the effects of stranding and isolation caused by water level fluctuation. Chironomidae, Oligochaeta, and pupating Trichoptera appeared to be unable to migrate with drop in water level.

The efficiency of a modified Ekman dredge was compared with that of an Orange-peel dredge. Analysis showed that a significant number of chironomids were lost when the Orange-peel was used in soft bottom types. A modification of the canvas flap on the Orange-peel is being designed to reduce this loss.

Aquatic insects have been collected with a #10 mesh net attached to the fish hatchery water supply pipe (this pipe draws water from the mud-water interface of the reservoir). Chaoborus, pupating Chironomidae, and pupating Trichoptera have been recovered with this

net in far greater numbers than with either plankton tows or bottom samples. Therefore, plans are being drafted for the construction of a modified Frolander-Pratt bottom skimmer which will quantitatively sample the mud-water interface for both aquatic insects and plankton.

## Currents

Current studies were conducted through the ice in February with a Price current meter. With the exceptions of the channel and upper section of the reservoir, water movements were generally too slow to be recorded with this instrument.

A more suitable method for measuring currents of low velocity utilizes a recording fluorometer and Rhodamine B dye. Preliminary tests were conducted using this method. These investigations indicated that spot and gravity-flow releases of the dye were not practical for mapping currents over distances more than 500 feet.

To overcome this limitation a fuel-pump feeder for releasing the dye is anchored in the reservoir and operated continuously for 24 hours prior to the start of tracing operations. With this procedure, currents may be mapped over distances of one mile or more. This method will be utilized next summer.

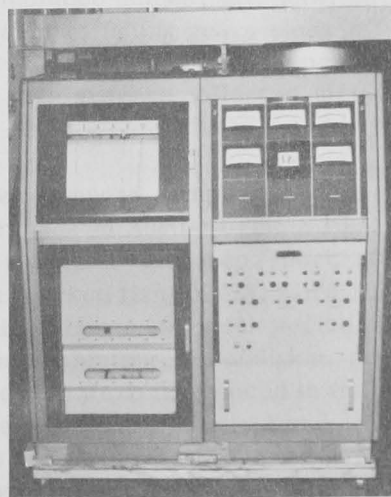


Figure 2:--Water quality monitoring system installed in Gavins Point powerhouse.

A water quality monitoring system has been installed recently in the powerhouse (figure 2). This instrument monitors water temperature, dissolved oxygen, pH, conductivity, and turbidity on a continuous basis. Attempts will be made to correlate these data with biological processes occurring in the reservoir. Data collected with the water quality monitor will also be compared with similar measurements taken throughout the reservoir.

Flooded trees

A graduate student from the University of South Dakota began a study on the effect of submerged trees on biological production in Lewis and Clark Lake and in Fort Randall reservoir. Artificial log substrates, 2 feet long and 8 inches in diameter, have been located at 2 stations to determine the ecological succession of plants and animals. Bottom fauna and plankton samples are being collected within flooded forests and in control areas. Flooded trees to be studied include emergent, submergent within euphotic zone, and submergent below euphotic zone.

A combination storage, laboratory and shop building was completed in January on the grounds of the Gavins Point National Fish Hatchery.

Plans for establishing and staffing a research station at Oahe Reservoir were prepared. Office and laboratory space will be in the U.S. Army Corps of Engineers administration center in the powerhouse.

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# SOUTH CENTRAL RESERVOIR INVESTIGATIONS

Fayetteville, Arkansas

Thomas O. Duncan, Chief

## HIGHLIGHTS

New office space was obtained in January, four additional biologists recruited by July, major field equipment and supplies procured by August, shop and warehouse spaces obtained in September, and field studies begun in late June.

A mark and recapture fish population estimate conducted on the 400-acre Beaver Reservoir dead storage basin indicated high "preimpoundment" numbers of young-of-the-year gizzard shad, black crappie, carp and largemouth bass.

Trap nets, gill nets, bottom trawls and two electrofishing units were extensively tested in Bull Shoals Reservoir during the second half of the year. SIMRAD echograms revealed fish concentrations at 30 to 50-foot depths which presents new sampling problems. A scaled-down version of the British Columbia herring trawl (10-foot footrope) has been constructed for sampling reservoir midwaters.

Extensive exploratory limnological sampling was conducted on Bull Shoals Reservoir to determine the minimum number of stations required to yield an adequate portrayal of physico-chemical conditions and plankton and bottom fauna production on a year round basis. Echo-sounder traces were made at major stations in an attempt to correlate fish distribution and movement with observed limnological conditions.

A creel census study design was developed which could provide reliable estimates of Beaver Reservoir sport fishing pressure and harvest during the early years of impoundment.

A two-man, dry submarine was procured, safety and underwater communications gear installed, and preliminary test dives conducted in Bull Shoals Reservoir.

A two-year preimpoundment contract study of the White River watershed above Beaver

Dam conducted by University of Arkansas faculty members and graduate students continued. Included are studies on White River fishes, bottom fauna, fish parasites, algae, fungi, and bacteria, and watershed soils and vegetation analyses.

Data collection and literature reviews were begun on life histories of the principal White River reservoir fishes. Research will be concentrated on reproduction, first year survival and growth, food habits and behavior of basses, crappies, clupeids, silversides, gars, and catfishes.

## POPULATION DYNAMICS

A modified, specially reinforced, flat-bottom boat was equipped and placed in service for bottom and midwater trawling. It is 22 feet long, with an 8 foot beam, a cabin near the bow and about 85 square feet of open deck space. Basic equipment includes a motor-driven winch, heavy pipe-frame and trawl block, tachometer, and a 90 HP outboard motor.

At Beaver Reservoir, a standing crop estimate was made in the 400-acre dead storage pool during the summer. Equipment used in the study included seines, gill nets, barrel traps, trap net, electrofishing gear, block-off net and rotenone. Forty-three species, predominantly of stream origin, were collected during the summer.

Two rotenone samples, each 2 acres in area were made in August using a 1 1/4 inch stretched mesh, 30 feet deep, block-off net. Caudal fin marked fish (6,278) were released in the basin during a two week period prior to the rotenone sampling. In addition, pectoral fin marked fish were introduced in the rotenone sampling areas (701 in the first and 350 in the second) to assist in estimating the population size in each rotenone sample area. The population estimates in rotenone samples were more reliable than the general mark and recapture results, and revealed an exceptional spawn of



black crappie during the year. Estimated densities of black crappie per acre were: entire pool, 2,800; first rotenone sample, 32,000; second sample, 3,065. The population of adult black crappie was probably very small since few were captured and none was recovered in the rotenone samples. Other species in order of abundance were gizzard shad young-of-the-year, carp young-of-the-year, and largemouth bass. Spotted bass, smallmouth bass, white crappie, white bass, walleye and channel catfish appeared in the samples.

A 25-foot and a 16-foot semi-balloon bottom trawl were tested with a 40 HP outboard motor. Bottom trawling provided some samples, but the scope of the operation was limited by submerged standing timber and the steep, rough terrain of the lake bottom. Attempts made to use the 16-foot semi-balloon trawl in midwater by adding depressors and floats were generally unsuccessful. Although the extremely rough terrain provides limited opportunity to use the otter trawls, drags which were completed without snagging produced good samples of bottom-dwelling fishes.

Other gear fished included gill nets, trap nets, and electrofishing equipment. Echo sounding demonstrated that large concentrations of fish occur in the mid-water regions of Bull Shoals during daylight hours. The conventional gear tested to date samples only when fish are distributed on or near the bottom. Beginning in October, efforts were directed toward mid-water sampling through development of a scaled-down model of the British Columbia midwater herring trawl developed by Barraclough and Johnson (1956). This trawl appears to have the characteristics essential for a sampling method which must be used in this reservoir. A small scale trawl (80 feet long) with doors on pennants was constructed and a series of tests begun.

Two trapnets were fished in several locations in Bull Shoals Reservoir from September through December. Catches have been below expectations. Although a large number of species were represented in the catch, none was taken in large numbers. With the advent of spring spawning activity it is anticipated that increased fish movement will increase catches in trap nets.

Catches in a few bottom-set gill nets were also small. Plans for future gill net fishing include the use of experimental Latin square design nets suspended in the mid-water zone.

The boat-mounted, electrofishing gear has proven to be both efficient and effective in shallow water, especially when used at night.

Catch per unit of effort, species composition, and lengths, weights, scale samples and sex data have been obtained for fishes taken with each type of gear.

The modified white-line echosounder of Norwegian manufacture was extensively field tested. The sounder is rugged, highly reliable, and easy to install and operate. It is powered by a 12-volt battery and consists of recorder, electronic elements, waterproof housing and transducer. The recordings give an excellent graphic presentation (figure 1) from about 10 feet below the surface to the bottom, depicting fish, trees, and small objects lowered in the water from the boat. The primary problem in applying the echosounder to a "population census" is centered around interpretation of the echo traces. Is a small trace one fish, or a school; If one fish, what size, etc? Used in conjunction with the two-man submarine, SCUBA divers, the midwater trawl, and possibly underwater photography, the traces should provide some quantitative measures of numbers and relative sizes of fish.

A cooperative Federal-State creel census program was designed for Beaver Reservoir. The study would be an invaluable adjunct to the planned reservoir research program as it would provide a measure of fishing pressure and harvest during the early years of impoundment. Knowledge of the angler's catch and effort, and related facts derived from a creel census would greatly enhance interpretation of research findings as well as establish levels of angler success for future comparison. Consideration is presently being given to this proposal by the concerned Federal and State agencies.

## LIMNOLOGY

A specially designed 20-foot flatbottom boat was equipped with pulleys, winches, orange

peel dredge, a modified SIMRAD echosounder, and electrofishing equipment. Original factory modifications included special reinforcement of the transom and sides for accomodating equipment, and the installation of two wells, one for the motor, and one for the echosounder transducer. After shakedown cruises, the two wells were closed, due to water turbulence interference and unsatisfactory steering performance.

Beginning in late June, more than forty stations were tentatively established for vertical profiles of dissolved oxygen, temperature, conductivity, pH, turbidity, and major chemical elements; and for samples of bottom fauna, plankton and fish populations. Data summaries were prepared and a minimum of four primary stations and 10 to 12 secondary stations selected on Bull Shoals to provide an adequate picture of environmental conditions.

Net plankton samples were taken at 14 stations on Bull Shoals Reservoir from June through December. A slide-mounted reference collection and checklist of plankton were prepared. Quantitative analysis was begun to determine relative abundance, distribution, and seasonal changes of plankton within the reservoir.

The physio-chemical data collected in 1963 indicates that Bull Shoals Reservoir is a complex system. The lake is 82 miles long and approximately 45,000 acres in size. The lower reservoir displays "orthograde" characteristics with well defined stratification (thermocline 30 and 50 feet), high dissolved oxygen, and high transparencies (15 to 30 feet). The mid-lake region (channel) is an intergradation of the lower and upper reservoir types. The upper reservoir is more river-like or eutrophic in nature with sedimentation more pronounced, transparencies less (5-9) nutrients and fish more abundant, and summer oxygen depletion below the thermocline.

Limited physio-chemical data collected in the dead storage pool of Beaver Reservoir during the summer revealed summer surface temperatures as high as 90° F while bottom temperatures were 57° F at 22 feet. Oxygen

depletion occurred below 12 feet. Transparency ranged from 1 1/2 to 4 feet.

## LIFE HISTORY

A comparative study was undertaken of the parasites of the fishes of Bull Shoals Reservoir and of the White River in the Beaver Reservoir basin. The problem was limited to examination of the parasitic fauna of 100 specimens each of the largemouth bass, smallmouth bass, spotted bass, green sunfish, and longear sunfish from Bull Shoals Reservoir. To date, 80 largemouth, 55 smallmouth, 90 spotted, 83 green sunfish, 100 longear sunfish, and 70 bluegills collected with electroshocker have been examined.

Thirteen years after impoundment the smallmouth bass of Bull Shoals Reservoir appear to be as heavily infested with Proteocephalus ambloplitis as any reported in the literature, yet the tapeworm is absent or rare in bass from unimpounded White River habitats. In comparison with the smallmouth, infestation of largemouth and spotted bass is moderate and infestation of green and longear sunfish is light.

Several rainbow trout stomachs from the lower section of Bull Shoals Reservoir were examined. The fish weighed from 1 to 3 pounds and were caught by anglers at 20 to 50 foot depths. The contents of 19 stomachs consisted primarily of phantom midge (Chaoborus) larvae which rarely appeared in midwater plankton and bottom dredge samples.

Data collection and literature reviews were initiated on the life histories of the principal White River reservoir fishes. Field activities will be concentrated on reproduction, first year survival and growth, food habits and behavior of basses, crappies, clupeids, silversides, gars, and catfishes. Lengths, weights, scales (spines for catfishes, and branchiostegal rays in gars) were collected for most of these groups. Additional specimens were collected for stomach analysis.

Preliminary examination of brook silver-side (Labidesthes sicculus) stomachs indicated zooplankton to be the principal food. Over 200

specimens were collected and placed in 10 percent formalin. Milky colored material was later observed in the bottom of the jar and upon examination was found to be zooplankton regurgitated by the silversides when placed in the formalin. Examination of scales from 20 specimens, 82 to 97 mm. in total length, indicated that all were in their first year of growth.

## GENERAL

The first six months of 1963 involved moving to new quarters, recruiting staff, completing the final project descriptions and procuring equipment. Beginning in late June, efforts were concentrated on developing, testing and modifying equipment, orientation of staff biologists and collection of basic data on the fish populations and limnology of the two reservoirs. Primary effort was placed on Bull Shoals Reservoir, and secondary effort on the small pool in Beaver Reservoir.

Talks were given during the year to community groups regarding the aims of the reservoir research program. The two-man submarine was part of the Bureau's display at the dedication of Greers Ferry Dam (figure 2).

Two warehouses were leased for storage of boats, trailers, nets, and other equipment, and construction and repair of nets.

## UNIVERSITY OF ARKANSAS CONTRACT RESEARCH

The Beaver Reservoir preimpoundment study under contract to the University of Arkansas, is aimed at quantitatively defining some of the factors influencing productivity in a reservoir during its early years. Research effort has been concentrated on soils and water, woody and herbaceous vegetative cover, forest floor mulch, bacterial action on submerged woody materials, aquatic invertebrate fauna, fish population abundance and distribution, and fish parasitism.

This year encompassed the second field season for all projects. Since October, emphasis has been placed on laboratory examination of samples, analysis of data, and prepara-

tion of final reports. A summary of preliminary results follows:

Bottom fauna:--Qualitative measurement of the adult aquatic insect fauna in the reservoir basin, using nets and light traps, continued. Studies of White River and tributary bottom fauna were complicated by drought conditions. Seasonal variations in relative abundance of major insect orders were marked, probably as a result of emergence patterns. Dipterans and ephemeropterans made up 60 to 70 percent of the populations sampled.

Watershed soils analysis:--Findings indicate a close relationship between geologic and soil derivation and the chemical composition of stream waters in the Beaver Reservoir watershed. Waters rising in sandstone-shale are of low pH, alkalinity hardness, calcium and magnesium. As the White River continues its flow north, it enters a cherty limestone region, and its waters become harder and more alkaline. It then enters an area of dolomitic limestone which contributes magnesium to the water. Percentage proportions of the alkalies in the river are: calcium - 73.1, sodium - 16.4, magnesium - 6.1, potassium - 4.3. Other constituents present were silica (5-10 ppm) boron (0.4 ppm), iron (0.2 ppm), aluminum (0.1 ppm), manganese (trace), and copper (trace). Soil and water samples on file will be analyzed by X-ray spectrographic methods for several trace elements: Mn, Cu, Zn, Ni, Mo, Co, Pb. It is possible that similar analysis of phytoplankton could define uptake of minor elements by these organisms and make possible prediction of their requirements.

Microbiology studies:--Qualitative and quantitative studies of algae, fungi, and bacteria in the watershed continued. In an attempt to define the contribution of drowned woody vegetation to new reservoir fertility, samples of wood and bark from 45 common woody plants were immersed in water for six months. After immersion, 0.1 ml. of the liquid was incorporated into 10 ml. of mineral salts media. Chlorella, Chlamydomonas, and Nostoc were employed as test organisms. Results varied but the water from most of the wood showed some stimulation of algal growth.

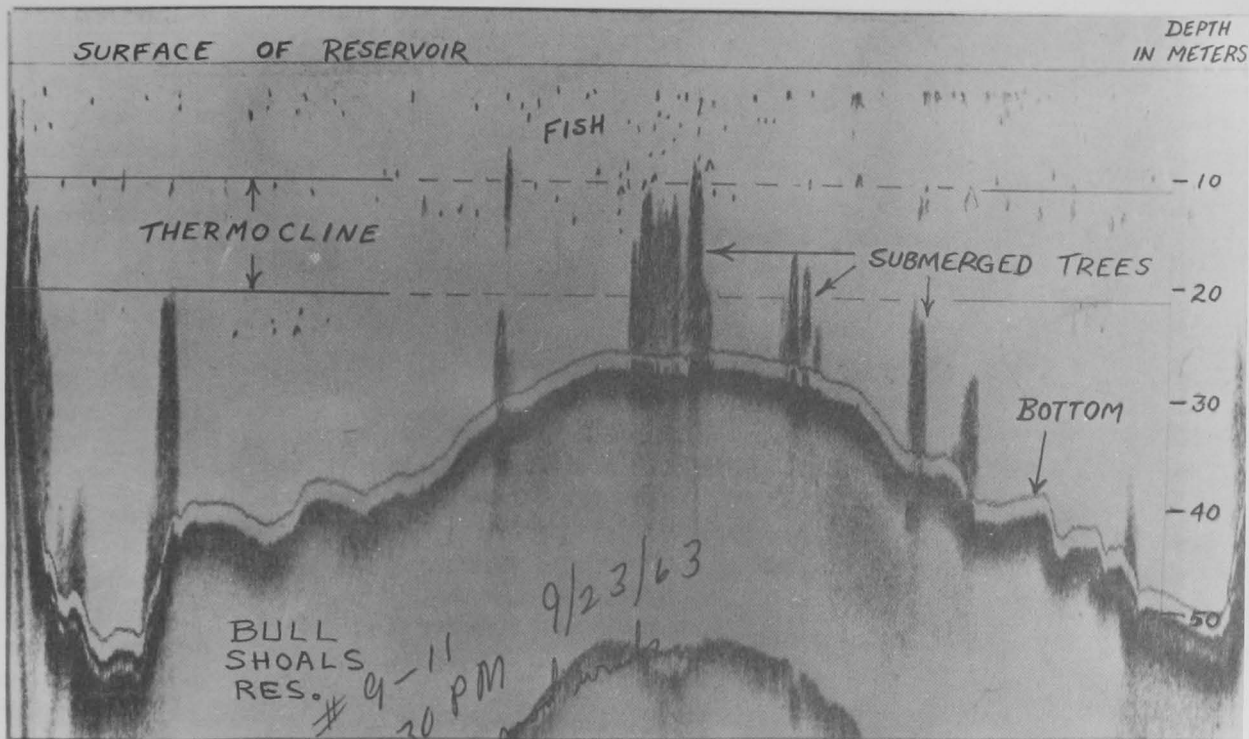


Figure 1:--An echosounder trace from Bull Shoals Reservoir in the vicinity of the confluence of the Little North Fork River (known as Theodosia Arm). The White River channel is obvious on each side of the echogram, with the channel on the left being downstream from the one on the right.



Figure 2:--Preparing to fish the 16-foot semi-balloon trawl.





Figure 3:--One of the two similar electrofishing rigs. The one pictured uses a 230-volt A.C., three-phase generator with three flexible electrodes.

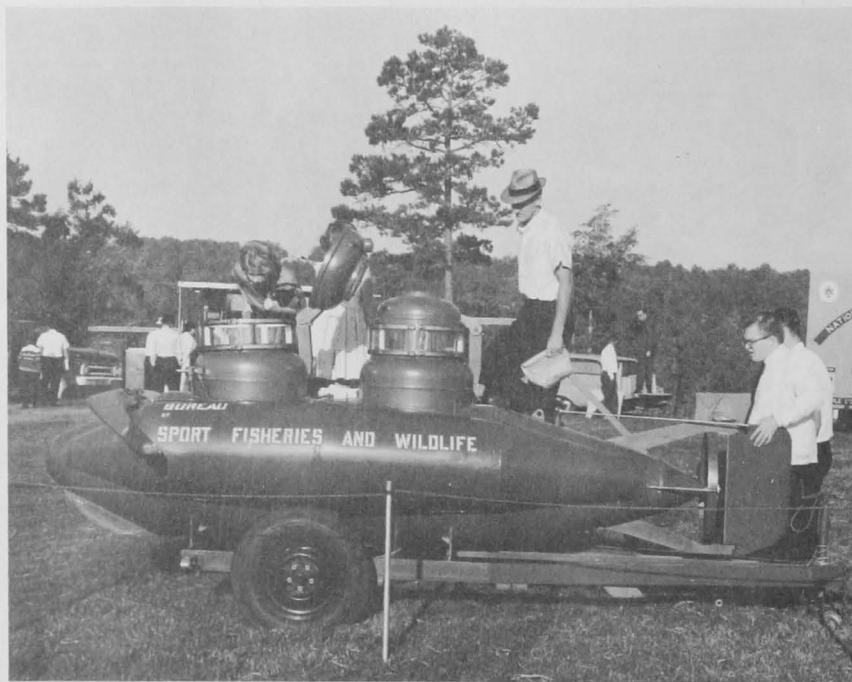


Figure 4:--Two-man dry submarine on display at Greers Ferry Dam dedication, October 3, 1963.



Watershed vegetation study:--Within seven main forest types, basal area of trees over one inch in diameter varied from 99 ft.<sup>2</sup> per acre in an elm-ash floodplain site to 57 ft.<sup>2</sup> per acre in a cedar glade. Density varied from 1,050 trees per acre in an oak-hickory site to 471 in a cedar glade. Mulch on the forest floor varied from 11,200 pounds per acre in a cedar glade to 2,010 on a willow gravel bar. Herbaceous understory vegetal cover measurements taken in August varied from 12 to 66 percent. Weight of herbaceous species varied from 190 pounds per acre in the poverty grass type to 17,050 pounds in the cane (Arundinaria) type.

Stream fish study:--Seventy-three collections, using electro-seine, bag seines, gill nets and cresol in the White River headwaters and tributaries, produced 73 species during an 18-month period. The relative abundance of species, correlated with various habitats and stream gradient, established definite patterns of distribution. The population structures of the headwaters, spring-fed areas and downstream, low-gradient stretches were strikingly different.

Fish parasite study:--Spotted, smallmouth, and largemouth bass in the watershed were all found to harbor the following parasites: Posthodiplostomum minimum, Clinostomum marginatum, Proteocephalus ambloplitis, Neoechinorhynchus cylindratus, Illinobdella moorei, Achtheres micropteri, and Spinitectus carolini. Camallanus oxycephalus, Contracaecum spiculigerum, and Lernaea cruciata were found in the largemouth bass only; Cystobranchus verrilli in the spotted bass only and Leucerothrus micropteri in the spotted and smallmouth bass only.

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Mr. James W. Mullan, Fishery Biologist  
Mr. Louis E. Voegelé, Fishery Biologist  
Mr. Richard L. Applegate, Fishery Biologist  
Mr. Horace E. Bryant, Fishery Biologist  
Mrs. Frances E. Nelson, Clerk-Typist

# MARINE

## SANDY HOOK MARINE LABORATORY

Highlands, New Jersey

L. A. Walford, Chief

### HIGHLIGHTS

Trials in the laboratory and field have demonstrated that bluefish can be tagged successfully, that they survive the tagging operation and travel considerable distances afterwards.

The problem of distinguishing populations of bluefish is being attacked by several techniques, including analysis of body proportions, growth rates, blood chemistry, and parasite content.

Sharks were less abundant in the middle Atlantic area in 1963 than in the two previous years. Thanks to enthusiastic cooperation of a large number of anglers who are particularly interested in shark fishing, we were able to tag 230 sharks.

Aerial survey of surface water temperatures in the Middle Atlantic area with infrared thermometer was continued monthly and aroused interest among marine scientists and anglers.

Experimental culture in our microbiology laboratory is approaching a point where we can use one species of microorganism (a dinoflagellate) for measuring and analyzing certain components involved in the biological productivity of sea water.

Experiments with several substances (detergents, soaps, chemosterilants), which are actual or potential pollutants of estuarine waters, have measured effects on survival and condition of estuarine fishes and invertebrates.

### NATURAL HISTORY OF GAME FISHES

#### Bluefish

Tracing migrations by tagging experiments:--Among several types of tags tested on bluefish, including Peterson disc, plastic loops, and darts, first in the laboratory pool and then

in the field, the best results were obtained with nylon inserted beneath the second dorsal fin brought into a loop and held at the ends with a friction V-clip. Returns from the three types used in field tests were as follows:

TAG TYPE	COLOR	NO. USED	NUMBER OF RETURNS	PERCENTAGE OF RETURNS
Jaw Loop	purple	136	1	0.7
	green	136	1	0.7
Dart	red	113	1	0.9
	blue	113	1	0.9
Dorsal Loop	purple	135	5	3.7
	green	134	5	3.7

Of the 767 bluefish tagged in field trials, 595 were tagged in the Sandy Hook area, 150 in the Long Island Sound area ("the Race"), and 22 at Cape Hatteras. Of the 14 returns 11 were from New Jersey tagging, 2 from Long Island and 1 from Cape Hatteras. Movement of bluefish eastward from northern New Jersey in mid-August is indicated from the pattern of recovery of tagged fish.

Bluefish population differences:--The last annual report described a theory, derived from seasonal catches in relation to inshore temperatures, that although the bluefish species is composed of several populations, two predominate over all others along the U.S. Atlantic coast. According to our theory, these winter in the south, probably in Florida, and migrate northward in early spring toward spawning and feeding grounds. One population ("A") centering during summer in the New Jersey-New York area, the other ("B") in the North Carolina-Chesapeake Bay area. The "A" population seems to be associated with water temperatures of 60° to 70° F.; the "B" population with temperatures of 70° to 80° F.

If this theory is correct, we would expect fish collected simultaneously from North Carolina and New Jersey to differ anatomically and physiologically, as well as in their migratory patterns. Accordingly, we have sampled bluefish in both

places, as well as in Florida and Virginia to compare anatomical features and growth.

Analysis of data is in progress. Preliminary results indicate that in certain body proportions, scale size and growth rate, samples from New Jersey differ significantly from those of North Carolina.

Parasites as indicators of races:--Herbert Anderson, working at the University of Miami, has completed a parasitological inventory of 438 bluefish, collected in New Jersey, North Carolina, and Florida. Analysis is in progress to determine how the species of parasites, numbers by area and time of occurrence, may relate to distinct populations of bluefish.

The following parasites were identified: (Trematoda) Microcotyle pomantomi, Lecithochirium branchialis, Proserhynchus crudiculum, Cymbophallus vitellosus, Bucephalopsis arcuatus, Stephanostomum tenue, Brachyphallus crenatus, Distoma fenestratum, Lepocreadium pyriforme; (Nematoda) Ascaris sp., Ichthyonema globiceps; (Copepoda) Lernanthropus pomatomi, Caligus schistonyx, Lernaenicus longiventris; (Cestoda) Nybelinia bisulcata, Callitetrarhynchus gracilis, Scolex pleuronectis, Otobothrium crenacolle, Synbothrium filicollé, Trypanorhyncha sp., Pseudotobothrium dipsacum; (Acanthocephala) Serrasentis socialis, Rhadinorhynchus tenuicornis, Echinorhynchus proteus, Echinorhynchus incrassatus; (Isopoda) Livoneca ovalis. Five parasites believed to be undescribed were recovered from bluefish. These included an encysted trypanorhynchid cestode and a haplobothrium cestode, an acanthocephalan, a nematode, and a digenetic trematode belonging to the family Paramphistomidae.

Blood chemistry:--Bluefish blood collected from 3 areas in New Jersey and one in North Carolina was analyzed in a preliminary study now in progress to assess the feasibility of using chemical characteristics for separating populations. Serum samples were pooled by sex and by size in order to obtain sufficient serum for complete analysis. Each sample represented an average of three fish, and all

samples were analyzed utilizing photoelectric colorimetry.

## Sharks

Survey in Middle Atlantic Bight:--Despite widespread attention focused on sharks as hazards to swimmers, liabilities to resort communities, nuisances to commercial fishermen, and more recently as important game fish, we know almost nothing of their habits in the open sea. This lack of fundamental information is a major obstacle to programs directed toward any of several practical application, i.e., controlling populations, developing repellents and protective devices, producing useful shark products, assessing beneficial and destructive effects of sharks as predators, and promoting sharks as a food and game fish resource.

In 1961 Mr. Casey undertook a biological survey of sharks in coastal waters of New York and New Jersey in an effort to determine species composition, abundance, seasonal occurrence, food and habits of large species in this region. Based on the first season's operations, which revealed highest concentrations of sharks on the "Mud Hole" grounds, 8 miles east of Sandy Hook, efforts during 1962-63 were concentrated in a study area (about 200 square miles) between Sandy Hook and western Long Island. Field work during 1961-62 covered the period from late August to early October, and in 1963 from mid-May to late September. Standard sampling procedure has been to fish 70 to 100 hooks of longline hooks for two hours at stations throughout the study area. Routine handling of sharks includes taking 30 morphometric measurements, tooth and vertebral counts, samples of skulls, jaws, vertebral columns, skins and parasites, and examination of stomach contents and gonads. Specimens not brought on board were tagged and released for migration studies.

This year between May 15 and September 20 Mr. Casey took 85 sharks representing 6 species on 51 longline sets. The most abundant large species represented were dusky (22) and sandbar (16), followed by white (9), mako (3), and tiger sharks (3). Also, 32 smooth dogfish were landed, and 66 specimens of large sharks

Longline catch of large sharks in New York - New Jersey coastal waters, 1961 - 1963.

Total catch for all areas

Comparison of catch in study area  
(Aug. 14 - Sept. 24)

Year	Date	No. of sets	No. of large sharks	No. of sets	No. of hooks	No. of large sharks	Average catch/100 hooks	Average surface temp.
1963	5/15-9/20	51	53	15	1182	6	.49	64.8
1962	7/15-10/11	33	91	11	1082	66	7.29	70.2
1961	8/23-10/12	38	244	12	1592	138	8.93	75.5

Species represented in three-year catch: sandbar (Carcharhinus milberti), dusky (C. obscurus), bull (C. leucas), tiger (Galeocerdo cuvieri), white (Carcharodon carcharias), mako (Isurus oxyrinchus), sand (Carcharias taurus), scalloped hammerhead (Sphyrna lewini), smooth hammerhead (S. zygaena), thresher (Alopias vulpinus).

were examined from catches of sport and commercial fishermen.

In the previous two years longline catches of large sharks during late summer and early fall were much higher than comparable periods for 1963. Only 4 of 41 sets in the study area this season produced over 3 sharks per 100 hooks. The notable exception was a catch of 19 sharks on an 80 hook set on August 1st. The decline in catch may be due to: (1) abnormally cold water temperature which prevailed during August and September; (2) a decrease in availability of prey species (catches of menhaden, bluefish and others were low during this period); or (3) effects of longline fishing in previous years.

While the catch of most species was low, that of the white shark was relatively high. Fifteen specimens taken from the study area (9 on our longlines, 6 by commercial fishermen) is the largest number reported from anywhere in the Atlantic during a single year. The size range of white sharks examined this year (4'9" to 7'8") and the presence of umbilical scars on two specimens indicate these were 0-1 year old individuals. Their relative abundance inshore suggests the nursery grounds for this species extend into shallow water where adults are rarely taken.

Although the summer months have long been recognized as the period of shark abundance in the northeast, detailed information for individual species is fragmentary. Our longline catches show the abundance of sandbar, dusky, hammerhead, and tiger sharks is highest in New Jersey waters between August 5 and September 24. During 1961 and 1962, catches in the study area declined sharply after September 20. During 1963 no large sharks were landed after September 9.

Migration studies:--Beginning in June 1,000 tags were sent to 50 volunteer sportsmen from Maine to Chesapeake Bay. By the end of summer 230 sharks had been tagged and three had been recovered, of which two were blue sharks from Montauk area and one a sandbar shark from Delaware Bay. The number of days between times of tagging and recovery were 9, 15, and 1, respectively; the maximum distance between points of tagging and recovery area was 25 miles. Species most often tagged were blues (132), dusksies (38), sandbars (31), and makos (8).

Results of the initial phase of the study are encouraging, particularly since availability of sharks was evidently low this year in many areas. The growing interest among sportsmen



to cooperate in the program has encouraged us to continue and expand these studies during 1964. Casey has designed tags for this purpose.

#### Observations of fish in their natural habitats

During the summer Mr. Wicklund and seasonal aides conducted field trials to test the feasibility of observing fish systematically in their natural habitats. Equipped with SCUBA, hookah gear and cameras, working in relays day and night, the group made several dives about favorite fishing grounds in the New Jersey area, notably the Shrewsbury Rocks and the Acid Grounds. To afford protection from large sharks which frequent these waters, Wicklund constructed a large steel cage within which the divers made their observations. Experience in 1963 will be the basis of formulating specific problems for study in the cage in 1964. Among significant observations made by Wicklund and his group were: juvenile bluefish congregating under floating objects, and young bluefish drifting at night in the water in a vertical position, apparently asleep. Anglers cast large quantities of ground fish - "chum" - into the water, most of which sinks to the bottom. There, Wicklund saw that fluke, blackfish, cunners, and various crustaceans consume it promptly. Among fishes residing at Shrewsbury Rocks, fluke occupies the top position in the hierarchy of what seems to be a peck order among species.

#### Analysis of fish movements from tag returns and contest records

Striped bass: - Analysis of sportsmen's catches from Schaefer Contest records of large (15 lbs. and greater) striped bass from the Middle Atlantic and New England areas were completed with help from the American Littoral Society's Scholarship apprentices.

Seasonal patterns similar for the three years, 1960, 1961, and 1962, suggest that the fishing season begins in April in Long Island Sound; that fish then appear to move out, proceeding westward along Long Island's south shore and northward along southern New England shores. New Jersey and Delaware fish move out of rivers from May to June, proceeding northward, and, joined by fish from areas

to the south, move into Long Island Sound and north to New England. They return south in the autumn, being caught in large numbers along the coast of southern New England, the south shore of Long Island and the New Jersey coast.

With assistance furnished through the American Littoral Society analysis was made on 506 returns from 11,000 striped bass tagged during the years 1959 to 1963 by sportsmen (sponsored by the Long Island League of Salt Water Sportsmen).

Recaptures of tagged fish were reported from St. Johns, New Brunswick, (Canada) to Chesapeake Bay. The tagging, although concentrated in the New York coastal area, includes enough activity from New England to New Jersey to provide a reasonable representation of the stocks of striped bass fished there by anglers.

Of the 506 bass tagged in all seasons only 22 were recaptured in Chesapeake Bay, 16 of these in winter, 6 in early spring. Of the Chesapeake recoveries, 5 were tagged in late summer, 17 in autumn. Recoveries by areas of tagging were as follows: from fish tagged in Rhode Island, 4; Long Island Sound, 3; New York Harbor and Hudson River System, 3; Long Island South Shore, 7; New Jersey, north of Barnegat, 5.

Of all recoveries 48 were in winter, 16 (one-third) Chesapeake Bay and 32 (two-thirds) from northward as far as New Brunswick. The pattern of winter returns suggests that most stripers, summering along the coast from New Jersey to Rhode Island, winter over north of the Chesapeake, particularly in Delaware Bay. The breakdown of winter returns is given below for stripers tagged from Rhode Island to New Jersey (Gulf of Maine tagging excluded):

<u>AREA</u>	<u>NO. OF WINTER RETURNS</u>
Chesapeake	16
Delaware-Maryland shore	4
Delaware Bay	16
New Jersey Coast	7
Hudson River	2



Bluefish:--Records on bluefish indicate that maximum angler's catches of large blues (4 lbs. and up) progress from south to north, consistent with a general movement up the coast during the season. Changes in average weight of fish caught from spring to fall during 1961 and submitted as contenders may reflect seasonal growth and also varying availability of different size groups.

### Physiology of fishes

Hormone induced spawning:--Maintenance of laboratory brood stocks of healthy, disease-resistant strains of marine game fish species with subsequent growth and reproduction of progeny has never been successful, despite efforts by laboratories throughout the world. With increasing destruction of estuarine spawning or nursery areas by human activities, artificial propagation of some marine species will probably become important in future conservation programs. Consequently, experimental rearing of marine fishes to establish a scientific basis of such programs is part of the long range plan of the Sandy Hook Laboratory.

The use of hormones to accelerate spawning of freshwater fishes has been successful, and permits investigators to breed selected specimens months and in some instances even years before normal sexual maturity would be attained. Dr. Eisler's group has been experimenting with human chorionic gonadotropin (HCG) and thyroid stimulating hormone (TSH) on bait fishes.

Results of preliminary studies indicate that a single dose of HCG (225 IU, IP injection) will measurably stimulate gonadal production in Fundulus in 7 to 10 days. Other experiments with TSH (0.2 unit IP injection) and HCG (200 IU, IP injection) in combination and singly, will produce accelerated gonad development in both sexes of Fundulus after 8 days. In all studies, control groups were injected with physiological saline. Further studies are indicated but experiments involving larger fishes such as flounder and striped bass are contingent on increased holding facilities and additional personnel.

Blood chemistry:--Dr. Eisler, Mr. Deuel, and summer aides, analyzed various components in whole blood and serum from samples of several marine fish species to lay the groundwork for gathering systematic data on blood characteristics of fishes. These data will be useful for reference purposes in further, more detailed studies to distinguish populations. Whole blood analyses included blood sugar, blood chloride, hemoglobin and red blood cell number.

### ENVIRONMENTAL RESEARCH

#### Study of fish distribution in relation to hydrography

As with most migratory species, bluefish distribution is evidently determined by internal processes such as a "biological clock" mechanism, temperature tolerances, conditioning, as well as external influences such as temperature, light, configuration of bottom, concentration of food, etc. To develop a hypothesis relating distributions to temperature, we are assembling all available records of temperature observations pertaining to the continental shelf from Cape Cod to the Florida Keys. We are preparing from these data a series of three-dimensional charts, showing minimum, maximum, and median temperatures from surface to bottom over the past 50 years at tri-monthly intervals.

Now far advanced, this study will probably be completed by summer.

#### Middle Atlantic survey

Observation of sea surface temperature by airborne infra-red thermometer, inaugurated in 1962 and described in the last Annual Report, was continued in 1963, through the cooperation of the U.S. Coast Guard. John Clark, making monthly flights, prepared charts of surface temperature fields from Montauk Point to Delaware Bay. These charts, covering the Middle Atlantic area, have aroused widespread interest in the United States and abroad, as evidenced by the large number of requests by anglers, news writers, and scientists, to be added to the mailing list. Moreover, other laboratories have

acquired instruments and have undertaken to cover sectors of the coast. Thus, the Sandy Hook Marine Laboratory, the Virginia Institute of Marine Sciences, the Bureau of Commercial Fisheries' Beaufort Biological Laboratory and the Gulf Coast Research Laboratory, are cooperating in a program of systematic, synoptic aerial surveys of surface temperature fields over the Continental Shelf extending from Montauk Point, Long Island, to Cape Fear, N. C., and the northern coast of the Gulf of Mexico.

With improvement in collection and reporting of anglers' catch and the localities of capture, relations between distribution of game fish and sea temperature should become better understood.

#### Biological assay

Gonyaulax scrippsae as a tool for analyzing Biochemistry of sea water:--Isolated in unialgal culture from a luminescent bloom in Sandy Hook Bay during September, 1962, Dr. Prager and Mr. Mahoney have established Gonyaulax scrippsae in pure culture and considerable progress has been made on understanding its biochemical requirements, bloom capabilities, and life history. This alga, which has generated considerable interest among scientists working on bioluminescence, is a potential tool for microbiological assay of sea water for vitamins B<sub>12</sub> and Biotin. This potential can be realized when its nutritional requirements are understood on a quantitative level.

Physical factors in algal productivity:-- An enclosed biologically and chemically defined environment provided a means of assessing effects of the physical environmental factors on algal productivity. Dr. Prager, with Richard Losick, and undergraduate National Science Foundation fellow from Princeton University, continued plastic bag studies begun in 1962, comparing the productivity of identical defined systems set out to sea with duplicates kept under varying conditions in the laboratory. Variations in photoperiod, temperature, and agitation were evaluated using cell number, cell size, and chlorophyll "a" content as criteria of productivity. Experiments were re-

peated in duplicate using cultures of Platymonas subcordiformis and Porphyridium cruentum, a green and a red alga, respectively.

Vitamin content of marine mud:--To account for the higher marine phytoplankton productivity associated with land masses, we are investigating the biochemical characteristics of inshore environments with Columbia University's Lamont Geological Observatory. American Littoral Society divers collected samples of marine mud for Drs. Burkholder, Gold, and Prager.

Samples are being assayed for vitamin content in an effort to understand subtle biochemical factors that trigger massive seasonal blooms of phytoplankters. This successful pilot study has established the practicality of employing amateur naturalists to collect in the field, samples suitable for biochemical analysis in the laboratory.

#### Culture collection

Stock cultures of the following species are maintained and can be sent to interested scientists upon request:

Monochrysis lutheri  
Isochrysis galbana  
Prymnesium parvum  
Platymonas subcordiformis  
Bodo caudatum  
Katodinium dorsalosulcum  
Gyrodinium intratum  
Gonyaulax tamarensis  
Glenodinium hallii  
Glenodinium foliaceum  
Gonyaulax scrippsae  
Unidentified Gymnodinium (designated  
"Claypit Creek isolate")  
Porphyridium cruentum  
Melosira sp.  
Nitzschia closterium  
Nitzschia punctata  
Chaetoceros sp. (5-10 u).

A variety of unialgal diatom cultures of some 20 common brackish water species are also maintained. In turn, contributions to our collections are most welcome.

## Fish kill studies

Mass mortalities of fish, mostly menhaden, but including other species, such as sea robin, occur in the Navesink and Shrewsbury Rivers and Sandy Hook Bay every year during late spring or early summer. These episodes are associated with immense quantities of dead plant material, mostly fragments of sea weeds, which sometimes accumulate so thickly that an outboard wake appears chocolate brown. This year, a fish kill occurred from June 25 - 29 in Sandy Hook Bay and patches of brown water persisted well into July. A hot drought throughout early June resulted in unusually high water temperatures (26 -28° C) and salinity (26 - 27 0/00) in Sandy Hook Bay. In the rivers, beds of attached algae died and broke loose and the tidal flush carried the fragments, with their associated diatom and protozoan communities, into the bay. The oxygen demand of such a decaying mass must have lowered the oxygen considerably ( no equipment was available to measure this), at least in the upper three feet of the bay in which the mass floated. The only possible toxin producer among the associated microorganisms which Prager identified was a dinoflagellate, Amphidinium fusiforme, but this was not in bloom numbers, and because attempts to isolate it in pure culture failed, it is not possible to confirm that it does produce toxin (variations in toxin production are known to occur among isolates of a single species). Other dominant microorganisms were identified.

We will study fish kills at their next occurrence and will gather more precise field data. Hypotheses to be considered are: (1) that the masses of plant material cause asphyxia in fishes either by clogging their gills or by decreasing dissolved oxygen below a critical threshold; and (2) sufficient dinoflagellate toxin is present to kill fishes.

## POLLUTION STUDIES

Effect of synthetic detergents on estuarine organisms:--U.S. sales of household synthetic detergents during 1962 were close to 4 billion pounds with sales increasing by 4 per

cent annually. This quantity of detergent represents about 560 million pounds of alkyl benzene sulfonate (ABS), a compound which is said to be relatively unaffected by sewage treatment or biological organisms. To date the effect of synthetic detergents on estuarine fishes has been ignored, although estuaries are essential to the normal growth and development of many valuable game fishes, including striped bass, winter flounder, and mullet. To evaluate the influence of a representative ABS-type synthetic detergent on various species of estuarine fishes, laboratory experiments were conducted by Dr. Eisler and Mr. Deuel. The following is a summary of their experiment to date:

Acute toxicity of representative household detergent (Tide) to five species of juvenile estuarine fishes was determined at 23 0/00 salinity and 20° C. Concentrations that kill 50 percent of the test organisms in 96 hours ranged from 7.0 ppm for silverside, Menidia menidia, to 22.5 ppm for mummichog, Fundulus heteroclitus. Intermediate in susceptibility were mullet, Mugil cephalus (10.1 ppm), winter flounder, Pseudopleuronectes americanus (8.2 ppm), and eel, Anguilla rostrata (7.5 ppm). The detergent remained toxic even after 12 weeks in solution. Alkyl benzene sulfonate (ABS) content of the detergent, as determined by the methylene blue method, was 30.3 percent.

Salinity of the ambient medium effects toxicity of the detergent. Acclimatized eel and mummichog, exposed to 10 and 20 ppm detergent, respectively, were relatively unaffected at low salinities (i.e. less than 20 0/00) but died at salinities approaching full ocean strength.

Lower concentrations (0, 1, 2, 5, 8, or 10 ppm) of packaged product at salinities of 10, 20, 30, or 40 0/00 for 150 days did not affect significantly any biological variable measured for juvenile Fundulus (i.e. mortality, growth in length and weight, red blood cell number, gonadosomatic index, liver condition).

On the basis of these experiments, it is concluded that relatively large amounts, such as one pound per 23,000 gallons of water, of ABS-type surfactant detergents are deleterious

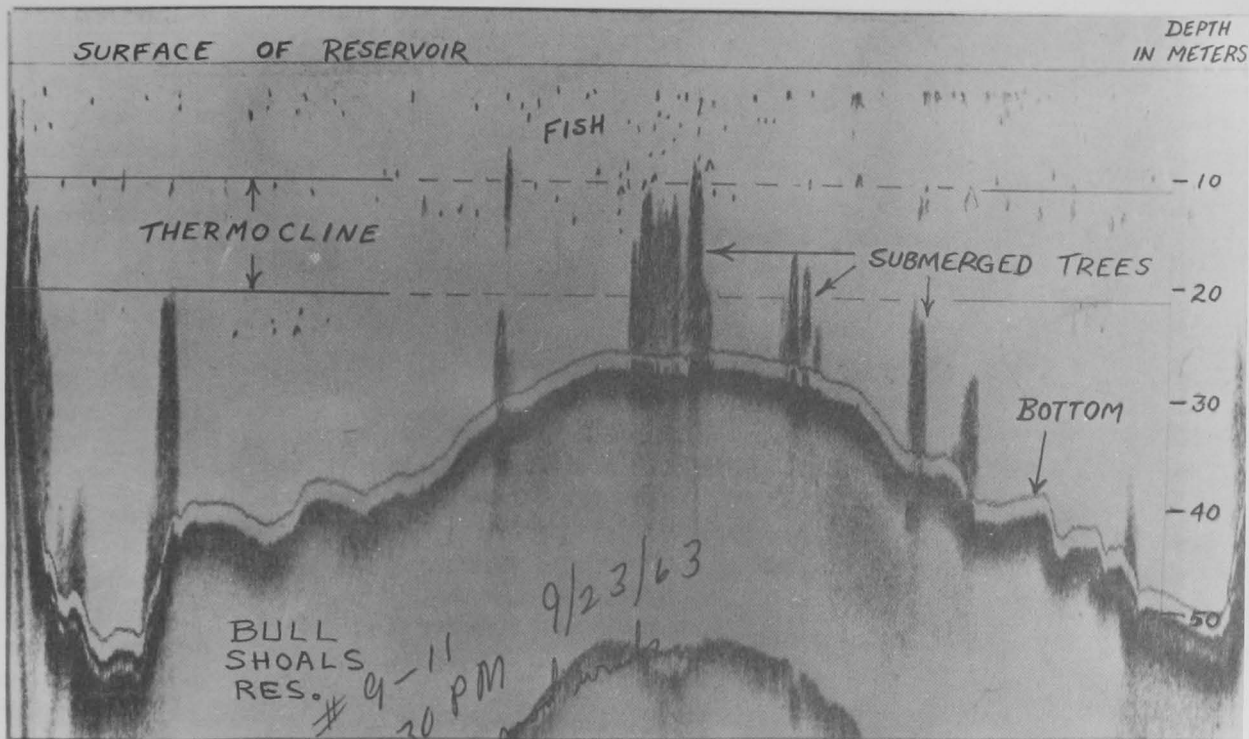


Figure 1:--An echosounder trace from Bull Shoals Reservoir in the vicinity of the confluence of the Little North Fork River (known as Theodosia Arm). The White River channel is obvious on each side of the echogram, with the channel on the left being downstream from the one on the right.



Figure 2:--Preparing to fish the 16-foot semi-balloon trawl.



## GENERAL

New facilities:--We are developing plans for an extension of the Atlantic Marine Game Fish Research Program to be housed on the campus of the University of Rhode Island Graduate School of Oceanography at Narragansett. A Providence firm of architects is designing a building which will be well equipped for experimental studies and will serve as a base for field studies.

The north wing of the Sandy Hook Marine Laboratory will shortly be remodelled to house tanks for experimental studies on behavior of marine game fishes. We expect to have this installation completed in time for use by summer of 1964.

The CHALLENGER was greatly improved through addition of 110 V auxilliary power, a new winch and remodeling to provide increased after-deck. A larger vessel was obtained on indefinite loan from the Army. Now being re-activated and outfitted it will be ready for sea duty in mid-April. This ship, designed as a sea-going tug, is virtually brand new, having never been put in service since she was built in 1957. She is steel, 400 gross tons, 107 feet in length, and has a 1,200 HP engine, can cruise at 12 knots and stay at sea for 2 to 3 weeks. By accepting a ship on loan rather than by transfer, we obtained a ship's complete equipment from blankets to radar.

Dr. Walford served as chairman of the SEAS Committee, an informal group of biologists, physical oceanographers, and geologists, representing Atlantic and Gulf Coast laboratories, organized to foster cooperation for systematic and synoptic studies in waters of Atlantic and Gulf shelf areas; secretary of the NASCO Panel on the Marine Environment; a group formed to advise in developing the Atlas of the Marine Environment; member of the U.S. National Committee on the International Union of Biological Sciences; member of the Marine Sub-Committee of the International Biological Program; and member of U.S. Delegation to the United Nations Conference on the Application of Science and Technology for the Benefit of the Less Developed Areas.

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TIBURON MARINE LABORATORY  
Tiburon, California  
Gerald B. Talbot, Chief

### HIGHLIGHTS

Publication of Atlas of Eastern Pacific Marine Game Fishing created unusual interest and demand.

Observation of feeding behavior of leopard grouper indicates interesting relationship with herring.

Monthly temperature surveys of three areas of Pacific Coast in cooperation with U.S. Coast Guard resulted in issuance of monthly temperature charts.

Sea bed drifters were used successfully in determining bottom current in Monterey Bay.

Tagging program on marlin and sailfish was initiated.

#### Atlas of marine game fish

The Atlas of Eastern Pacific Marine Game Fishing was published during October. Requests for this publication are much greater than anticipated, and the original printing of 2,000 copies is exhausted, with over 3,000 requests on hand and more being received each day. Additional copies are being printed to meet the demand.

#### LIFE HISTORY AND ECOLOGY OF THE STRIPED SEA PERCH

Field studies on the striped sea perch (Embiotoca lateralis) were carried out at Yaquina Bay, with laboratory and library work centered at Oregon State University. Tagging experiments in the Bay indicated a territorial preference by sea perch. The spawning areas were found to be on and along the edges of eel grass flats near the mouth of Yaquina Bay. The spawning period appears to be from the last week in May to July 1. Mature males were found near the end of September, indicating that the mating season was approaching, but no

females with fertilized ovaries were found by the end of September when the field season was terminated. Data to determine age, food habits and economic importance are presently being analysed.

#### FEEDING AND SCHOOLING BEHAVIOR OF GAME FISH

Field research during 1963 was centered mainly on the leopard grouper (Mycteroperca rosacea) in the Buena Vista area of Baja California. Limited tagging studies indicated that each rocky area had a resident population, and there appeared to be no movement from one rocky area to another. Herring (Harengula thrissina), when present, appear to be a major food item of the grouper. The herring move inshore in the early morning, over the rocky areas, then spread out along the beach. The groupers follow the herring inshore about sunrise, and after a period of concentrated feeding begin an offshore movement which peaks between 8:30 and 9:30 A. M.

In the afternoon the groupers begin another inshore movement which peaks between 4:30 and 5:30. The number of groupers

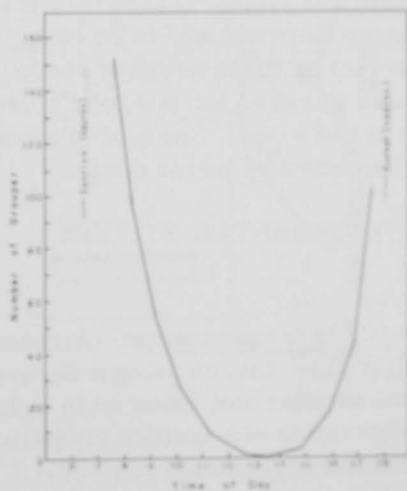


Figure1:--Average number of groupers counted under herring school near Buena Vista, California, at various times during several days of observations.

increases until the herring move offshore, beginning about 6:00 P.M. (sunset at this time was about 5:45 P.M.) Peak feeding of the grouper on herring occurred at this time (figure 1).

The herring leave the shallow beach area over the same rocky route of the inshore migration, not straying over the sandy areas on either side. When the edge of the rocks is reached, the herring swim in a large milling school before continuing out over the sand into deep water.

A study was conducted in the Buena Vista area of the relative position of 21 species of fish at midday as contrasted to midnight. A diagrammatic sketch was completed showing the position of the fishes in relation to their environment during the two periods. Several of the fish were found to be "asleep" at night and could be handled gently without being awakened.

The two-man research submarine was used in Baja California for one month for study on the leopard grouper. While in this area, a complete test of the vehicle was carried out. Many construction details of the submarine left something to be desired, but most can be corrected with minimum difficulty. It became apparent that the submarine could be operated efficiently in the field only if a supporting vessel capable of lifting the sub aboard for maintenance and repairs were available. Otherwise, it is difficult to charge batteries and to do other maintenance. Two or three hours of shore maintenance was required for one hour's use of the vehicle in the water. Some of this could probably be eliminated by better design.

## ENVIRONMENTAL STUDIES

Sea-surface temperatures:--Airborne infrared temperature studies using a Barnes 14-312 infrared sensing unit mounted in light aircraft, to determine sea surface temperatures were continued in early 1963.

Data were analysed on the effect of atmospheric moisture (haze) upon the instrument's temperature readout. Water droplets in a density near that of a solid overcast can result in the reading of cloud temperatures.

Twenty-two consecutive observations were made at altitudes of 500 feet, 1,000 feet, 1,500 feet, 2,000 feet and 3,000 feet over a designated area of San Francisco Bay. Temperature readings from the airborne infrared unit and the water surface temperature as determined by a mercury standard thermometer were compared. Results for atmospheric conditions existing on the day of observation indicate an average deviation in the airborne readout of  $-0.34^{\circ}$  F. at 500 feet,  $-0.36^{\circ}$  F. at 1,000 feet,  $-0.50^{\circ}$  F. at 1,500 feet and  $-0.75^{\circ}$  F. at 2,000 feet (figure 2). The haze level extended from the surface to 2,000 feet. Average deviation in the relatively clear atmosphere at 3,000 feet was  $0.85^{\circ}$  F., only  $0.10^{\circ}$  F. more than that at 2,000 feet.

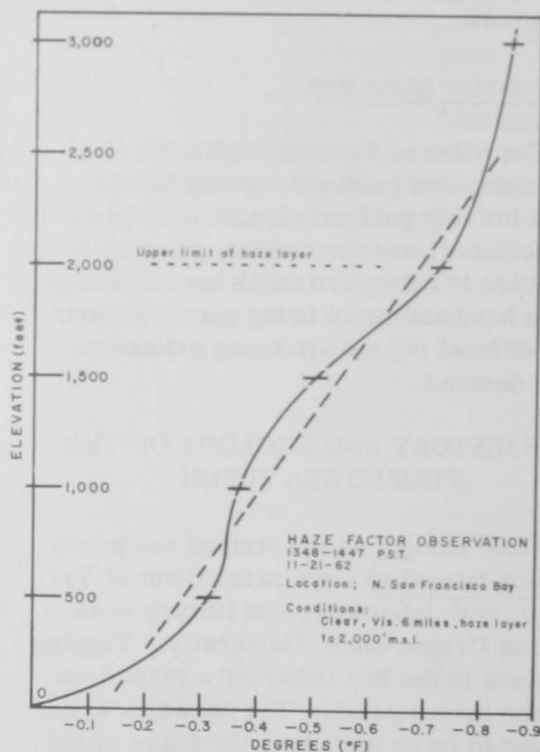


Figure 2:--Measurements on the effect of haze on water surface temperature readings as determined by an airborne infrared detector.

Synoptic airborne infrared temperature surveys were conducted:

1. For the area from North San Francisco Bay Delta and the Sacramento River;
2. For three index areas between Mexico and Cape Flattery, Washington;
3. In the vicinity of warm water outfalls of four steam electric generating plants in southern California.

Airborne infrared temperature observations were made once a month for the first four months in 1963 from Raccoon Straits, San Francisco Bay, to approximately 10 miles north of Sacramento on the Sacramento River. The survey of this 125 mile segment of the Sacramento River system reveals a correlation between temperature change and climatic conditions; the location of major temperature change, marine waters to fresh waters (due to tidal fluctuations), was easily observed.

During nearshore coastal surveys large increases in surface temperature were noted near the condenser cooling water outfalls of steam electric generating plants. These plants use large quantities of salt water, 300,000 to 450,000 gallons per minute, for cooling of steam condensers. The process usually raises the water temperature 18° F. to 22° F. Based on surveys of four plants in the southern California area made in January and February, isotherm patterns of the area surrounding the outfalls were drawn (for example, see figure 3). This was the first application of the airborne infrared technique to the measurement of temperature gradients around the outfalls. This method has since been used by three of the largest electric utility companies in California in their engineering research programs to design outfall systems that will reduce the amount of environmental modification.

Continental Shelf temperature surveys of three Pacific Coast areas were initiated in August 1963. The surveys are made in cooperation with the U.S. Coast Guard. Surveys are conducted off the coasts of Washington, northern

Oregon, and central and southern California (figure 4) to better understand the role of the ocean environment upon marine species. The synoptic track lines are 800 to 1,000 miles in length and require 6 to 7 1/2 hours of flight time to complete. This provides a near simultaneous record of sea surface temperatures.

After the completion of each survey, surface isotherm charts are drawn for duplication and distribution to interested persons and to oceanographic, fisheries, and meteorological laboratories.

Albacore are rarely caught in temperatures below 58° F. or higher than 69° F., with the majority of the catch being made in areas having water of approximately 64° F.

Temperature records from the September and October flights off San Francisco and the location of the Albacore fleet corroborated this relationship. Airborne temperature surveys thus offer a promising method for finding Albacore.

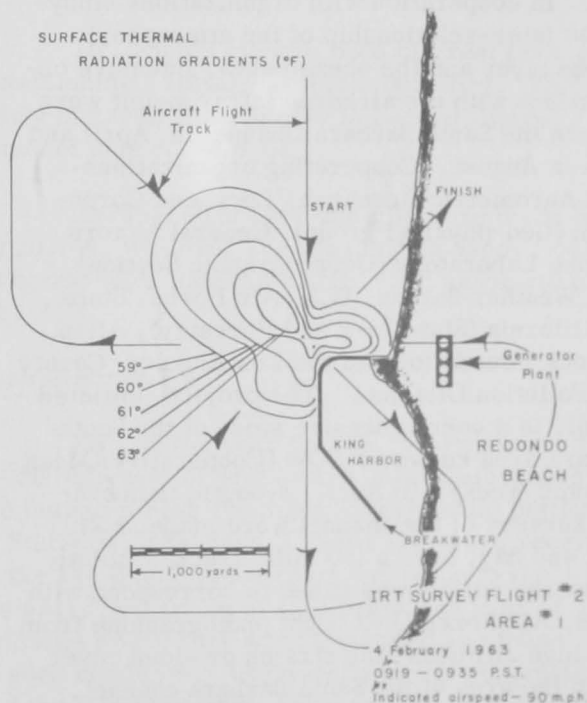


Figure 3:--Surface thermal gradients surrounding an electric generating plant cooling water outfall.

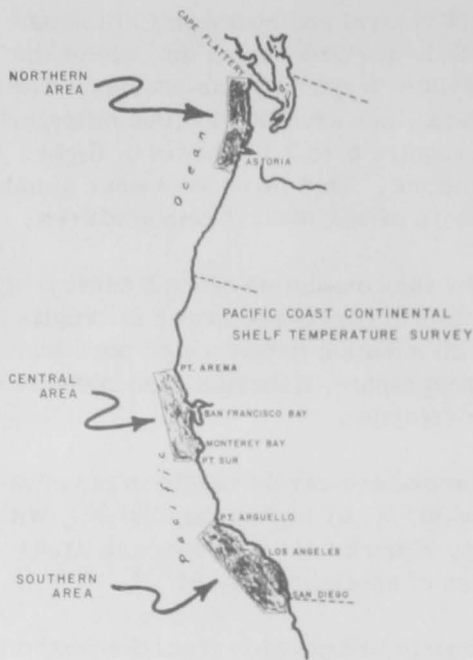


Figure 4:--Areas covered during monthly airborne infrared sea surface temperature surveys in cooperation with the U.S. Coast Guard.

In cooperation with organizations studying the inter-relationship of the atmospheric marine layer and the ocean below, intensive observations with the airborne infrared unit were made in the Santa Barbara Channel in April and again in August. Cooperating organizations were Aerometric Research, Lockheed Corporation (Geo-physical group), General Motors Defense Laboratory (Oceanographic Section), U.S. Weather Bureau, U.S. Air Force, State of California (State Park and Forestry), Allan Hancock Foundation and the Los Angeles County Air Pollution District. The project, initiated in 1961, is a comprehensive study of the Santa Barbara area known as COW (Cooperative Oceanographic Week). In April, synoptic temperature surveys of the channel were made, both night and day, over a 150 mile track. In late August the survey was timed to correspond with a U.S. Air Force U-2 flight photographing from very high altitudes any stratus or cloud cover within the area of the Santa Barbara channel. In addition, observations were made of air temperatures at 500 feet for the southern area.

### Catch-temperature relationships

Studies are underway to determine the relationship of sport fish catches to depth and temperature. Party boat operators have been furnished bathythermographs to record depths and temperatures at which each species is caught. While it is too early to predict results, it appears that there is a good correlation between the temperature, depth and catch of some species.

### Bottom current studies

In 1962 limited studies were undertaken to determine bottom current flow in Monterey Bay by the use of plastic sea bed drifters. Monterey Bay was selected as a test area for reasons of bottom topography, sandy shoreline, and because it is an important marine game fishing area. The initial airborne drop of sea bed drifters, made in cooperation with the U.S. Navy, tested a rapid method of distributing the drifters over a grid pattern. A second experiment was conducted in February 1963. The plastic sea bed drifters were slightly modified to prevent the loss of plastic tops due to impact with the slipstream of the aircraft. A packet of five drifters was dropped at each of 25 stations. Fourteen percent have now been recovered, and results show a counter-clockwise movement of bottom water.

### Surface current studies

In early 1963 a series of tests were carried out to determine whether the standard glass drift bottle is capable of withstanding the impact shock of water when ejected from aircraft flying at various altitudes. A small amount of fluorescence dye was added to the sand used in ballasting each bottle, and this proved to be effective in determining the utility of the bottle after water impact. It was found that results varied with size of bottles, but dye release increased with altitude of drop (500' to 2,000'). Tests comparing corked and capped bottles indicated that release of dye could result from either a breakage or uncorking.

Observations on pelagic schooling species were continued by four spotters currently operating off the California Coast from Monterey Bay to Mexico. The airborne fish spotters record observations, using small tape recorders, of all species seen during each flight, their location, and estimate of abundance. Species most commonly observed are anchovies, sardines, mackerel, barracuda, yellowtail, sea bass, bonito, sharks, rays and tuna. Tape recorded observations are transcribed to a storage reel at the laboratory; information is then coded for date, time, area covered during flight, location of fish species, number of schools or area covered by the school, estimate of school weight; also remarks are noted on such subjects as behavior and movements.

This experimental method is being evaluated for reliability.

#### COOPERATIVE TAGGING PROGRAM

The Pacific area tagging program for marlin and sailfish began near the close of 1963 with the tagging of 15 marlins and 8 sailfish in southern California and Baja California. This program is an extension of the Atlantic tagging program sponsored by the Woods Hole Oceanographic Institution and the International Game Fish Association. The Tiburon Marine Laboratory in cooperation with the other two organizations was asked to coordinate the Pacific activities. Tagging supplies are being furnished to anglers, charter boat owners and others who desire to land only record size fish. Smaller marlins and sailfish are tagged and released.

Several tag recoveries revealing spectacular transoceanic migrations have been made in the Atlantic where the program has been underway for several years. Despite the short period of operation in the Pacific area one marlin, tagged near La Paz, Baja California, was recovered 1,150 miles to the southwest by a Japanese fishing boat. 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.

#### Lease

The U.S. Navy has offered the Department of the Interior a 5-year lease on certain buildings at the U.S. Naval Net Depot for an Oceanographic Center which will include the Tiburon Marine Laboratory. Action on this lease is pending in Washington.

#### Library

Dr. Willis H. Rich, Professor Emeritus of Stanford University, donated his extensive personal library to the laboratory. Dr. Rich has had a varied and distinguished career in fishery science while working for the U.S. Bureau of Fisheries, Oregon Fish Commission, U.S. Fish and Wildlife Service, Administrator of Fishery Production, U.S. Department of the Interior, and was fishery advisor for several agencies in India, Indonesia and Taiwan. At present he is a consultant for the Corps of Engineers, U. S. Army.

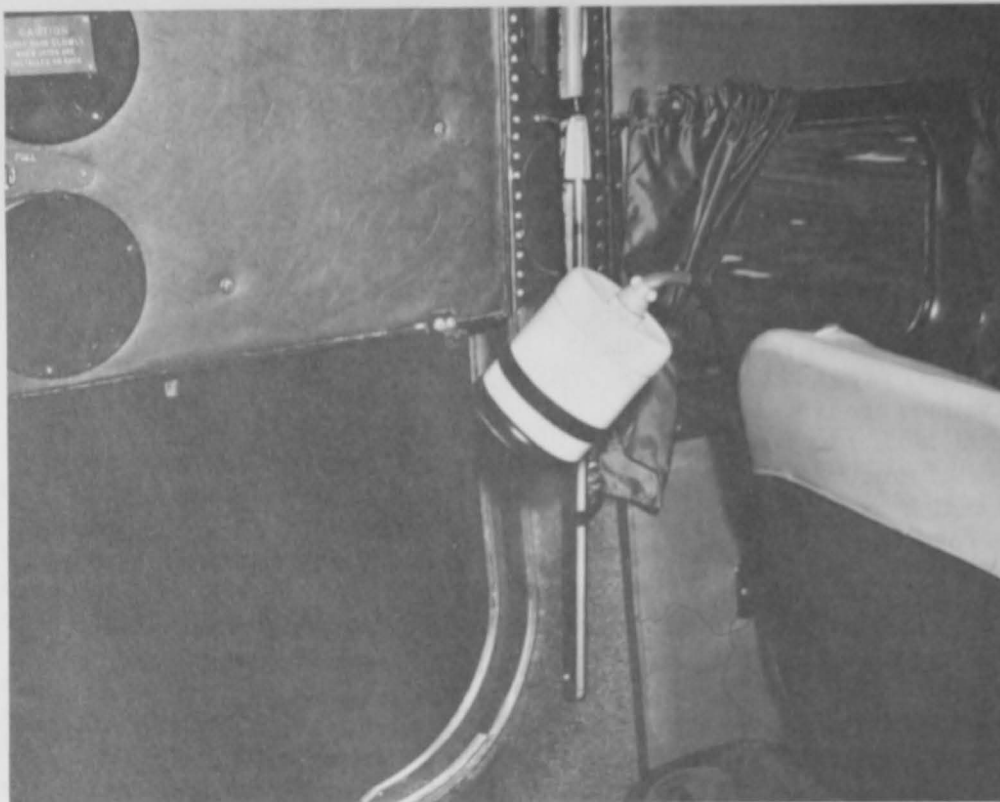
Dr. Rich's gift has been of great help in establishing a library at this laboratory. The early material particularly is needed, since much of it is out of print.

Arrangements for this gift were made by Dr. L. A. Walford, Director of the Sandy Hook Laboratory, a long-time friend and associate of Dr. Rich.

#### Fish shipment

Late in the year the laboratory assisted in the shipment of grass carp from Malaysia to the Bureau's Fish Farming Experimental Station at Stuttgart, Arkansas. These fish were received at the San Francisco airport and taken to the Steinhart Aquarium at California Academy of Sciences where they were examined and found in good condition. The water was re-oxygenated and the fish shipped to Arkansas where it was later reported they arrived without loss.





Infrared detector views the ocean surface through the lower half of the port hatch.



Infrared electronic console (left) and strip chart temperature recorder (right) positioned in the survey aircraft.



U.S. Coast Guard aircraft used in Pacific cooperative temperature surveys--U.S.C.G. UF-2 Grumman Albatross.

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Mr. Robert S. Kiwala, Fishery Technician

# TECHNICAL COMMUNICATION

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#### TRANSLATION

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