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UWFL-37

ACTIVITIES OF THE APPLIED FISHERIES LABORATORY
UNIVERSITY OF WASHINGTON, 1953-1954

Applied Fisheries Laboratory
University of Washington
Seattle, Washington

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Contents

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Introduction

The activities of the Applied Fisheries Laboratory for the 1952-1953 fiscal year were summarized in UWFL-34[2]. The programs and study areas described in that report have been continued in 1953-1954, with varying degrees of emphasis dependent upon the needs of the moment and the time and personnel available for the particular problem. The urgency for completion of the field studies undertaken at Eniwetok during the fall of 1952 and the planning and execution of a program for the spring of 1954 at Eniwetok and Bikini have required the major portion of the energy and thought of the entire staff. Other sections of the program had to be postponed in view of the urgency of the present field studies. This postponement will delay the Laboratory's basic program of measuring radiation effects--a program of experiments needed to assist in interpreting data in the applied fields.

Program as Defined by Contract

The contracts between the University of Washington and the United States Atomic Energy Commission define the areas in which the Laboratory works, but with the limited staff and facilities fields of emphasis must be subject to change as the occasion arises.

The areas of effort have been defined in the contracts along the following general lines.

1. To conduct studies on the immediate and continued effects of radiation and radioactive materials on living organisms. Aquatic life will be used for the most part, but fauna and flora existing adjacent to an aquatic environment also will be studied, as overlapping problems make such studies necessary.

   a. Laboratory studies of a fundamental nature on the effects of radiation and radioactive materials on aquatic life will be continued at the Applied Fisheries Laboratory.

   b. Field studies on the broad aspects of radiation and radioactive contamination of fauna and flora will be carried on at field laboratories where atomic bombs are tested, such as those at Bikini and Eniwetok Atolls.

2. Studies on the effects of radiation and radioactive materials will be conducted in cooperation with the Aquatic Biology Group, General Electric Company, Nucleonics Division, Hanford Works. Areas of mutual interest will be explored so as to use the unique facilities and personnel available at each of the Laboratories in a complementary fashion.

   Laboratory Studies--Effects of radiation and radioactive materials on aquatic life

   (a) Effects of ionizing radiation on various stages of development of salmonoids.

   (b) Effects of ionizing radiation on organisms other than fish

   (c) Experiments with chinook salmon eggs and young in water of various temperatures

   (d) Experiments planned with various racial stocks of salmon on conditions for spawning and reproduction

(e) Management programs for increasing salmon populations in the Northwest where depleted by toxic contamination

(f) Study of the effects of ionizing radiation on gametes of salmon

(g) Program of studies involving genetic problems in salmonoids

Field Studies--Radiobiological studies of the contamination of Eniwetok and Bikini

(a) 1954 spring series of weapons tests with the field portion of the radiobiological investigations starting in March and continuing, with constant observation, for twelve months

(b) Laboratory investigations involving uptake and biological cycling of radioactive materials

(c) Studies and plans for future Pacific tests and surveys

Cooperative Studies--Consultative and cooperative services with General Electric Company Radiological Sciences Department and the Hanford Operations Office

(a) Furnishing experimental stock for the Hanford Works aquatic biology program

(b) Final rearing and release of fingerlings exposed to pile effluent waters at the Hanford Works Aquatic Biology Laboratory

(c) Provision of facilities and services to accommodate return of Hanford effluent exposed salmon

(d) Other related services as requested by General Electric and agreeable to the University of Washington

(e) Services to the Commission through the Hanford Operations Office:

1. Training schools in radiation measurement

2. Information as requested

Program Studies on the Fundamental Effects of Radiation upon Aquatic Animals

During the fiscal year of 1953-1954 work in other areas and in field-work planning necessitated the curtailment of a portion of this program. However, progress was made in completing the work initiated in previous years and on current projects.

A. Biological effects of X-rays on invertebrates

The major current research deals with the lethal effects of X-rays upon the embryological development of the fresh-water snail, Helisoma. Documentary photomicrographs recording development of irradiated and control egg masses were reviewed and the results summarized for tabulation. Further irradiation experiments were conducted in an effort to reduce variability or experimental error. Eggs undergoing the first cleavage were stained in order to relate the mitotic phases to certain characteristic stages in the microscopic appearance of the cleaving egg. During the summer a report of this experiment will be ready for publication.

Early this fiscal year installation was completed on the beryllium-window X-ray unit, which expedited both radio-biological and roentgenographic experiments. Incidental to this activity a paper[6] on the radiography of small fishes for meristic studies was published in August 1953.

As indicated in UWFL-34[2] future work will include, in addition to snail egg studies, a comparison of the relative biological effectiveness of soft and hard X-rays and Co^{60} gamma rays using agar plates of _Chlorella_.

B. _Radiobiological studies of fishes_

Results of the experiment on the effects of X-radiation on early stages of development of salmonoids have been reported in UWFL-34. The report on the preliminary experiment on the effects of X-rays on six developmental stages of rainbow trout, _Salmo gairdnerii_, involving a total of approximately one thousand fish, is being written. Mortality, growth, body proportions, fin ray number and color (parr marks) have been studied and found to be altered by comparatively low doses. Teratological effects on external features are now being analyzed. This material will then be summarized and compared with radiation effects on other animals.

The second salmonoid experiment in progress on _Oncorhynchus kisutch_ (silver salmon) is a much more complex study on effects of X-rays on 17 developmental stages involving a total of approximately 11,500 fish. This study will include analyzing the skeletal system (by radiographs) developed subsequent to X-radiation as well as making observations similar to those made in the trout.

C. X-ray diffraction and electron microscope studies on silver salmon spermatozoa

In the X-ray diffraction studies of silver salmon spermatozoan tails, orientation of tails in a parallel configuration was done by (1) quick-freezing a flowing suspension of tails both on glass plates and in capillaries and (2) by orientation in an electric field.

In X-ray diffraction work with undried tail preparations, the use of antibiotics (penicillin G, streptomycin sulfate) resulted in marked agglutination effects, thus the steps for preparing pure suspensions of tails could not be accomplished. The use of benzene and toluene as antibiotics gave similar results. Osmic tetroxide at a concentration of .05 per cent has been tried with some success. Sufficient purified tail fibril material has not been obtained for extraction of actomyosin.

As a test for survival in cold storage, the spermatozoa were mixed with 10 per cent glycerol, cooled slowly to -5°C, then cooled rapidly to -79°C. The spermatozoa were found to be motile after thawing.

During the summer of 1953, electron microscope studies on silver salmon spermatozoa were made. An ultramicrotome and an improved RCA-EMU-type electron microscope provided by

Dr. H.S. Bennett of the Anatomy Department, University of Washington, was used.

Many sections 1/100 to 1/20 μ thickness were made with silver salmon spermatozoa for a continuation of an earlier study[5]. In the earlier work it had been found that the tail was composed of an isodiametric filament containing eleven fibrils and was covered by a spirally wound, membranous sheath. Nine of the fibrils are of equal diameter, forming a ring in cross section, and are each made up of five protofibrils. The remaining two fibrils are of smaller diameter, are located in the middle of the ring of fibrils, and contain four protofibrils each.

The new findings from sectioned material are as follows:

The spermatozoan head is covered with two double membranes between which are many mitochondrial granules. Both the membranes and granules are sensitive to changes in osmotic pressure (Fig. 1).

Both the proximal and distal centrioles are located well inside a pocket at the posterior end of the nuclear material. The more anterior proximal centriole is composed of globules in a ring configuration and is attached to the nuclear membrane. The distal centriole lies just posterior to the proximal centriole and is fused into the anterior ends of the nine-ring tail fibrils (Fig. 1). The central tail fibrils extend on through the distal centriole into the proximal centriole.

The neckpiece contains the greatest concentration of mitochondrial granules found in the spermatozoan (Fig. 2) and, in addition, a septal structure which may be the golgi remnant (Figs. 2 and 3).

The tail sheath is a double membranous structure surrounding the tail fibrils. It is spindle-shaped in cross section (Figs. 4, 5, and 6) rather than round as had been assumed previously. The sheath is semipermeable as evidenced by marked swelling with fixation in hypotonic solution.

Mitochondrial granules are found throughout the length of the tail between the sheath and the axial filaments (Fig. 4). The larger granules are composed of subunits and are never found with the ring formed by the ring fibrils.

In the tail fibrils evidence of substructure within each fibril is found in cross sections of the tails (Figs. 4, 5 and 6). There is no lateral association between adjacent fibrils, the diameters of which are not altered with changes in osmotic pressure.

D. Personnel

The studies for this portion of our program have required the services of about 25 per cent of the Laboratory's research staff.

Members of the staff assigned to this part of the project are:

Kelshaw Bonham	full time
Frank G. Lowman	three-fourths time
Arthur D. Welander	one-fourth time

Radiobiological Studies of the Contamination of Eniwetok and Bikini

Laboratory studies of a limited scope have been conducted at the Applied Fisheries Laboratory to support the field projects at the Pacific Proving Ground. These experiments include physical, biological and chemical studies.

A. Uptake of fission products by algae

Experiments on the fate of fission products ingested by crabs eating radioactive *Ulva lactuca* or *Nereocystis leutkeana* indicated that most of the activity in the crabs was found in the digestive glands (51 per cent), and that the feces contained 42 per cent of the activity over a period of 17 days. The remainder was present in the gills, muscles, and carapace.

During the year, reports will be written on the above experiments and on the work done with uptake of fission products from Engebi sand (1952) by algae.

Experiments with the green alga, *Chlorella pyrenoidosa*, to determine whether it absorbed the activity rather than adsorbed it onto its surfaces, were conducted. In 30 seconds incubation in "hot" solutions dead algae killed by heating or by trichloroacetic acid retained as much activity as did live algae; however, with increasing incubation time, the living algae accumulated much more activity whereas the dead cells accumulated none. The metabolism of the living algae, therefore, had something to do with the uptake of radioactive particles. From decay curves it appears that this activity could be due to one or more of the following isotopes: Ce^{144}, Ru^{106}, Zr^{95}, Cs^{137}, and Sr^{90}.

Radiochemical analyses of this material are in progress. The decay curves also show the same slopes in the algae as in the solutions, indicating no selective uptake by the algae. When Ce^{144} was added to the solution of mixed fission products, uptake by the algae was very rapid.

Other experiments with circles of the alga _Ulva_ _lactuca_ and _Nereocystis_ _leutkeana_, incubated in solutions of mixed fission products from Engebi sand, showed that five per cent of the total activity in solution was taken up per gram of _Ulva_ in 12 days and 10 per cent by _Nereocystis_ in three days. These experiments will be continued.

Electron micrographs of the filtered Engebi sand solution were prepared. No particles were found in suspension, showing that there was a true solution rather than a colloidal suspension of the radioactive particles.

B. Food cycling experiments with invertebrates

In the experiment with the water movement by the horse mussel, _Modiolus_ _modiolus_, the presence of suspended matter (carmine, or Aqua-dag) in the sea water was found to depress the pumping rate. Suspensions of the order of 0.001 per cent cause a reduction of about 15 per cent at 12°C. With heavy suspensions of carmine (0.1 per cent) the pumping becomes erratic with periods of one minute or less of more rapid rates than normal and periods of ten to thirty seconds of cessation of pumping. Pumping of 300 to 400 cc is followed by sudden closure with the forceful ejection of pseudofeces. Modiolus has been found to be extremely sensitive to vibrations; even the activation of sump pumps in the building could be detected by momentary cessation or depression of the pumping rate, although there was no accompanying shell movement. This was not fully appreciated at the time most of the runs on the effect of suspended material were made. These will consequently have to be repeated. No tidal rhythm was noted as has been reported for Mytilus. It is hoped these experiments can be resumed in July 1954.

Several local marine gastropods and the shore crab, Hemigrapsus nudus, have been brought into the laboratory and tested for suitability for cycling experiments with Chlorella and Ulva. Thus far only Hemigrapsus has been found to be sufficiently predictable in its appetite and easily manageable. Results of preliminary feeding experiments with Ulva are reported in the section on algae.

A survey of the literature concerning coral reefs and their invertebrate fauna has been made. Emphasis has been placed on papers dealing with ecology and physiology. Growth rates of corals as reported in the literature are difficult to compare because different criteria have been used. However, it is evident from several specimens of Pocillopora sp. which were collected during the 1948 biological resurvey of Bikini that there is a more rapid growth at Bikini--perhaps twice as great--than at other places reported. An effort will be made to obtain more reliable data of this nature at Eniwetok.

C. Radiochemical analyses

Radiochemical analyses were made of a number of soil samples and biological samples taken at Eniwetok Atoll in November 1952 immediately following the Mike shot. Selective absorption of fission product isotopes by plants and animals collected soon after the shot was not observed. Results of these analyses are tabulated in the Applied Fisheries Laboratory report UWFL-33[1].

Portions of soil from Rojoa, Runit, Rigili, Igurin, and Japtan Islands and contents of the intestines from sea cucumbers taken near Japtan, Rigili, Runit, Aaraanbiru, and Bogallua Islands were analyzed for uranium and plutonium content. It was thought that the contents of sea cucumber intestines could well be used as bottom samples, since it is known that sea cucumbers of that species ingest bottom material indiscriminately and that they migrate very little. Activity of approximately six disintegrations per minute per gram (d/m/g) dried soil was found in Rigili and Runit soil and 30 d/m/g in that from Rojoa, and from four to 200 d/m/g in the sea cucumber intestine contents. This work will soon be published as an Applied Fisheries Laboratory report.

Other projects included the development of a technique for separating trace quantities of radiostrontium from large amounts of ashed biological material or sand from the Pacific area and the analysis of well water used in the School of Fisheries experimental hatchery.

D. Studies of the Marshall Islands fishes

A good deal of work was done with fishes of the Marshall Islands in preparation for the studies at Bikini and Eniwetok this year. A field key for rapid identification at the Eniwetok laboratory of over one hundred species which are most abundant on the reefs was prepared and taken to the Pacific laboratory. Also, an improved fish sampling program was organized for the investigations at the field laboratories.

A paper on fishes of the Marshall and Marianas Islands[7], with one of the Laboratory staff members as co-author, was published by the Smithsonian Institute, United States National Museum, in November 1953.

E. Personnel

Planning for the spring series of tests at Bikini and Eniwetok has required the major portion (60 per cent) of the time of the entire staff during the fiscal year. The preparations for the tests and the early phases of the field study have been reported in USFL-35[3] and UWFL-36[4].

Members of the Applied Fisheries Laboratory staff assigned to this project include:

Edward E. Held
Ralph F. Palumbo
Dorothy J. South
Paul R. Olson
Frank G. Lowman
Arthur D. Welander
Lauren R. Donaldson
Allyn H. Seymour

We wish to acknowledge the services of Major Charles M. Barnes, USAF, VC, from March 11 to July 1, 1954.

Cooperative Studies

Some of the experiments at the Laboratory are conducted in cooperation with the Hanford Works, since the two facilities working together provide much of the desired equipment, location and experienced personnel necessary to the accomplishment of these experiments. Portions of the project are carried on in one laboratory for a time and then continued by transferring the animals to the other laboratory for further development. Parts B. and C. of this section are projects of this type.

A. Temperature experiment with chinook salmon

The third and final phase of the temperature experiment with chinook salmon eggs and was initiated in September. This phase of the work varied from what has been done previously in that four instead of one race of salmon were used and in that the temperatures changed with the season instead of remaining constant. Observations are being made of mortality, rate of development, occurrence of morphological abnormalities, growth rate, and variation in meristic characters both within and between racial groups. The experiment will be concluded the first of May. Tabulation of some of the data from the earlier experiments has been completed. Analyses of the remaining data and a report upon the entire experiment are expected to be completed during the next fiscal year.

B. Chinook salmon experiment--1949 brood year

During the fall of 1949, chinook salmon eggs were obtained at Green River Station (20 miles south of Seattle), Washington State Department of Fisheries. These eggs were transferred to the 146 Building, Hanford Works, where they were incubated and the young fish reared until June 1950, at which time the survivors were hauled in tank trucks to the Applied Fisheries Laboratory at the University of Washington. The fingerlings were held and fed in the pools at the University until July 1, 1950, when they were released.

During the incubation of the eggs and early feeding of the fish at 146 Building, Hanford Works, some lots were retained in dilute effluent water (five per cent) and a like number were reared in controlled river water. Before release the fish were marked, the former by removal of the right ventral fin, the latter by removal of the left ventral.

These fish were well represented in both the sports and commercial fisheries as is shown in the following table compiled from data collected by the Washington State Department of Fisheries. The actual recaptures are many times greater than the numbers recorded in the table since only a small percentage of the marked fish caught are reported to the Fisheries Department.

Since the catch in the fishery of the RV-marked salmon was considerably greater than that of the control salmon, it appears that survival, at least to the salt water stage, was not adversely affected by rearing the fry and fingerlings in five per cent effluent water. The failure of more than one salmon of this experiment to return "home" in the fall of 1953 was disappointing and unexpected, hence survival to maturity and possible genetic effects cannot be evaluated.

Number of Chinook Salmon Recaptured from 12,410 LV and 13,174 RV Marked Fry Released July 1, 1950, from the University of Washington

Year	No. Recaptured LV	No. Recaptured RV	Size in Pounds	Area	Remarks
1950	0	0			
1951	11	15	1-5	Puget Sound	Sports-fishermen
1952	29	54	2-11	" "	Sports-fishermen
	57*	44	6-18 1/2	Ocean	Commercial
1953	7	30	8 1/2-34	Puget Sound and Ocean	Sports-fishermen
	0	1		U of W	Trap
Total	47 to 104	144			

*Caught off the coast of Washington; may be from Kalama, Samish or University of Washington since a similar mark was used for all three.

C. Silver salmon experiment--1951 brood year

In the fall of 1951, experiments with silver salmon eggs and fingerlings were conducted following the pattern used with the 1949 brood year chinook salmon.

The silver salmon experiment was undertaken to provide additional information on the effect of dilute concentrations of the effluent water upon the fresh-water stage of one of the important commercial salmon inhabiting the Columbia River drainage. The use of silver salmon for such experiments has certain advantages over Chinook salmon, for the silver salmon have a longer fresh-water life, 18± months as compared to 6± for chinooks. Furthermore, silver salmon have a shorter life span, normally returning to spawn and die at the end of their third year rather than the fourth as in the case of chinook salmon. The expectancy of return to the home stream is higher for silver salmon, being about one per cent.

During the fall and winter of 1953-1954 five precocious males, "jacks," of each mark returned to the University of Washington ponds. Also some of this lot have been caught and currently are being caught in the sport fishery. The return of this lot as mature spawning fish is expected during the late fall months of 1954.

Other Services

In addition to the research program, the staff members of the Laboratory have provided various services to state, city and campus organizations. Numerous requests are received, for example, for lectures on atomic energy, advice on radiological problems, library service, loan of equipment, etc. The staff also assumed the responsibility for and served as members of the local Atomic Energy Commission emergency monitoring team.

Radiological problems that have arisen at the Boeing Company, the local civil defense organization and at other radiological laboratories on the campus have been brought to our staff members for discussion and advice. Also, our X-ray machines have been available to other departments on the campus.

Monitoring team

In the spring of 1950, when the United States Atomic Energy Commission voluntarily undertook the provision of civil defense emergency monitoring service, the University of Washington organized a fourteen-man emergency monitoring team, with Dr. Donaldson of the Applied Fisheries Laboratory as team captain. This group has been active for four years, meeting as scheduled, giving lectures, and providing technical advice and other services whenever called upon.

In a recent letter from Dr. David Shaw, Manager, Hanford Operations Office, the University was informed that through legislation of the Congress the Federal Civil Defense Agency has been charged with the responsibility for national civil defense planning and that the voluntary responsibility assumed by the Atomic Energy Commission for emergency monitoring has now been terminated.

Library services

The Applied Fisheries Laboratory library of selected unclassified research and development reports, besides supplying current scientific information and technical reference material for the Laboratory staff, is used also by University faculty members and students, from whom many requests for documents are received. The reports are checked out on a two-week loan basis or longer if they are needed for interlibrary loans for use by libraries on other campuses in the state.

Talks delivered by members of the Applied Fisheries Laboratory on the subject of atomic energy

Speaker	Date (1953)	Group	Location
L.R. Donaldson	July	School of Science Oregon State College	Corvallis, Oregon
L.R. Donaldson	Aug.	Exchange Club	Seattle
F.G. Lowman	Sept.	Kalama Men's Club	Kelso, Washington

Speaker	Date	Group	Location
	(1953)		
L.R. Donaldson	Dec.	Phi Delta Kappa	Seattle
	(1954)		
L.R. Donaldson	Jan.	Washington Institute of Chemical Engineers	Seattle
F.G. Lowman	Jan.	Seattle Civil Defense Organization	Seattle
L.R. Donaldson	Feb.	Thirteenth Naval District World Affairs Seminar	Seattle
F.G. Lowman	Feb.	Kent Lions Club	Kent, Washington.
L.R. Donaldson	April	Science Teachers of Shoreline School District	Seattle

Official Visitors at the Applied Fisheries Laboratory
July 1953 - January 1954

John C. Bugher	Washington, D.C.
Harold H. Plough	" "
George L. Glasheen	" "
Paul G. LeFevre	" "
Charles M. Barnes	Richland, Washington
Kenneth L. Englund	" "
Norman H. Brand	" "
Leo K. Bustad	" "
Richard F. Foster	" "
R.W. Wager	" "
Lynn A. George	" "
Royal E. Rostenbach	" "
Barbara Gillooly	University of Calicornia at Los Angeles
David S. Anthony	Miamisburg, Ohio

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3. Preliminary statement of the proposed program for Marine Survey Unit, Section 19. 1953. Applied Fisheries Laboratory, University of Washington. UWFL-35

4. Operations outline for Program 19, Marine Survey Unit, of Operation Castle. 1954. Applied Fisheries Laboratory, University of Washington. UWFL-36

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Numbers of days to hatching and absorption of yolk sac and per cent cumulative mortality of chinook salmon reared at city water temperature and various constant temperatures. Eggs from a single pair from Soos Creek spawned on November 15, 1951.

Lot and temp. °F	Number of days to		Cumulative mortality in per cent to				
	Hatching*	Abs. of yolk sac**	Hatching	Feeding	34th week***	46th week	Remarks
34°			100				None hatched; all dead after 156 days
40°	128	225	7	12	14	31	Did not feed; weight at 46th week less than at hatching
45°	80	145	6	8	8	11	
50°	51	97	13	17	22	23	
55°	41	81	5	86	86	87	High mortality during absorption of yolk sac
60°	35	73	22	99	100		Two survived to feeding; last died on 165th day (April 28)
62-1/2°	32		76	100			All died before absorption of yolk sac; last died on 50th day
65°	28		100				Greatest mortality about time of hatching; two completed hatching but died 2 days later (28th day)
67°			100				All died by 20th day
74°			100				All died within 3 days
City water temp. range	63 54°-42°	126 42°-47°	6 55°-41°	19 41°-47°	27 46°-63°	66 63°-67° 64°	Greatest mortality after July 4 when water temperature was above 63°F

*The day on which 50% of lot was hatched.
**Approximately the day on which all yolk sacs were abosrbed.
***First week in July.

Figures 1, 2, and 3. Longitudinal sections through the heads of silver salmon spermatozoa.

Figure 4. Sections of spermatozoan tails showing a longitudinal section (A), oblique sections (B), and a cross section (C). Substructure within the fibrils is shown in the oblique sections.

Figures 5 and 6. Cross sections through spermatozoan tails showing the spindle-shaped sheath and multiple unit fibrils.

mg - mitochondrial granule	dc - distal centriole
cs - cytoplasmic sheath	rf - ring fibril
ns - nuclear sheath	cf - central fibril
n - nuclear material	np - neckpiece
pc - proximal centriole	gr - golgi remnant

APPENDIX