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PROGRAM OF THE APPLIED FISHERIES LABORATORY,
UNIVERSITY OF WASHINGTON, FOR THE 1956 TEST SERIES
AT BIKINI AND ENIWETOK ATOLLS, MARSHALL ISLANDS

9

Applied Fisheries Laboratory
University of Washington
Seattle, Washington

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PROGRAM OF THE APPLIED FISHERIES LABORATORY,
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Introduction

The general program of study for the Applied Fisheries Laboratory, University of Washington, was outlined in UWFL-41. The proposals in Section C, page 21, of that report, "Studies of radiobiological contamination resulting from weapons tests in the Marshall Islands" have been re-evaluated following additional field studies in 1955 and a further development of the testing program. The program presented here, it is hoped, will form the basic development of the studies to be included in the next test program.

Monitoring Program

The Applied Fisheries Laboratory has conducted radiation monitoring studies in the Marshall Islands since the Crossroads tests of 1946. During this long series of experiments a background of information on methods and an understanding of the amount, distribution, cycling, and decline of radioactive materials in the area have been developed. It is proposed, therefore, in the present test series to limit the monitoring program to

specific measurements that will permit a general evaluation and thus release the field personnel and laboratory facilities for the study of special problems of interest.

A. Physical measurements

As in past experiments it is desirable to obtain the broad basic measurement of the relative amounts, composition, particle size, and non-fission components of the fallout material. To accomplish the study the following projects should be included in the physical measurements program.

1. Surface readings of radioactive contamination of the islands at 1-inch and 3-foot levels. These readings to be made at the same time the biological samples are being obtained.

2. Complete chemical separations of ~~fallout materials~~ with ~~qualitative~~ and quantitative evaluation of radioactivity from fission and non-fission product sources.

3. Determination of the physical ~~nature~~ of the fallout material using techniques similar to those described in DWFL-33².

4. Collection and evaluation of soil and lagoon bottom radiation content, similar to the studies of DWFL-43³.

B. Biological evaluations

The biological monitoring program should be one of adequate sampling to indicate orders of magnitude and trends of radiation contamination. By limiting the collections to certain forms and using only specific tissues more time will be available to develop other aspects of the program.

The monitoring program should be based at Eniwetok and samples collected during the operations at that testing site. A sampling location for monitoring can be decided upon after the sites and orders of magnitude of the tests are made known to the Applied Fisheries Laboratory personnel.

Pre-test samples of the biological materials and physical measurements to be used in monitoring should be obtained before the tests start. Subsequent samples for the entire series should be taken at monthly intervals to measure the rates of uptake and decline of activity. The entire program should be completed in about six months.

Bio-monitoring at Bikini and other sites would be considered "crash" programs and activated only if a specific need existed and a definite program were made.

1. Bio-monitoring of Fishes

Past experience has indicated that variation in the amounts of radioactive substance present in the tissues of fish is so great that fairly numerous samples (50 to 100) should be taken for a reliable average. Furthermore, the amounts of radioactivity present in each of the tissues sampled (muscle, bone, skin, liver and viscera) tend to bear the same relation or ratio to each other on a per-gram-wet-weight basis, so that estimates of the amount present in tissues not assayed may be made from the tissues assayed. It is suggested, therefore, in order to speed up the monitoring procedure that the following methods be adopted.

- a. Fifty to 100 fish should be obtained for a sample from a single area. The sample to contain the common larger forms of the reefs and neighboring open waters with more than five species involved, if possible.
- b. Fish should be frozen as soon after collecting as possible.
- c. Rate meter reading of whole fish using a probe should be made.
- d. Remove from frozen fish tissues of uniform size and shape to fit conveniently on a 1" to 1½" plate. It is important that the size and weight of the tissues be consistent.
- e. Tissues to be assayed will be muscle and liver, preferably from large fish so that dissection of frozen tissues will be facilitated to conform to (c).
- f. Using a rate meter plated wet tissues will then be analyzed for variation in the amounts of radioactive materials.
- g. All like tissues thus obtained will be placed in a Waring Blender and homogenized.
- h. Two or three samples of equal size will be removed from the Blender, dried in pliofilta bags and sent to Seattle for counting in the Nucleometers.

1. The rest of the sample will be dried and shipped to Seattle for chemical analysis.

From each area sampled, comprising 50 fish or more, 4 to 6 plates will be prepared for radioassay. The counts of the 50 or more wet samples from the rate meter will be averaged and the average compared with that obtained from counts made in the Nucleometer. The values thus obtained will be used to determine the variation present in the individual fish. The procedure, although unlike that of former analyses, is similar enough to make it possible to correlate results with past work.

2. Bio-monitoring of Invertebrates

The invertebrates, which make up such a large part of the life of the atoll and which play so important a role in the food cycles, will be included in the sample taken for bio-assay. The great variety of forms, however, make it necessary to limit the sampling to selected species and specific tissues.

- a. The land crab, or hermit crab, Geenobita perlatua, widely distributed on most of the islands, should serve as one of the forms used for bio-monitoring.

It is not necessary to destroy the animals. For sampling will be limited to the removal of one leg from each of three crabs. After taking the wet weight, the muscle from the legs should

be minced on the counting plate, dried and counted. The dried exoskeleton will then be sealed in a plastic bag for counting at the Applied Fisheries Laboratory.

- b. Beach crabs, or ghost crabs, Gerygone ceratophthalma, will be processed like the land crabs, except that the samples should contain two legs from each crab because of the small amount of muscle in each leg.
- c. The boring clam, Tridacna imbecilis, will be used for bio-monitoring the molluscs. Again it is not necessary to destroy the clams, as samples can be limited to the removal of about three grams of the mantle from each of three clams.
- d. In the sampling series the coral, Acropora, should be used, with collections made from the terminal portions of each of ten separate colonies. The terminal portions of the samples equal to one gram in dry weight should be crushed, plated and the radioactivity determined.
- e. Three specimens of sea cucumbers, Holothuria atra, should be collected. After washing the integument thoroughly and blotting it dry, three grams from each specimen should be minced on separate plates, dried and counted. Like samples of the gills and intestine content will be used also.

- f. Sponges should be sampled, with three samples from each of three specimens used for counting.

3. Bio-monitoring of Land Plants and Algae

As previous experiments have shown that there is very little difference in the levels of activity in the various species of land plants and algae, collections could be limited to three species of algae and three of land plants. Since in earlier studies there appeared to be no correlation between number of samples taken and amount of variability in the counts, the best method of sampling might be to lump several samples together and then take an aliquot to give an average value. Two aliquots should be enough for checking. Thus each collection or sampling would require 6 plates for algae and 18 plates for land plants (for each location).

Algae (ontire)

Halimeda-2
Caulerpa-2
Syringodium

Land Plants

Scaevola frutescens
Boerhaavia argentea
Plumbago procumbens
youngest leaves-2
internal stem-2
flowers or fruits-2

4. Bio-monitoring of Plankton

Evaluation of the radioactivity in plankton is important to studies of cycling of fission products in lagoon organisms, as plankton is the base of the food chain. Plankton is

also a good indicator of the presence of contaminated water and in the past has often yielded the highest counts per unit weight of all the organisms in the collection. For these reasons plankton tows should be made at the same time as the scheduled general collections for monitoring biological contamination.

"Crash" Program

During the past testing operations a number of "crash" problems have arisen. This type of problem should be realistically planned for in developing a program as extensive and varied as that planned for the spring of 1955.

Following the Castle test, at least eight problem areas developed which required evaluation and which occupied the time, energy and facilities of the Laboratory for the major part of the past year and one half.

To provide for unexpected problems that almost certainly will arise during the coming series, two or four of the Laboratory's senior scientific staff should be available at the ENEL for reassignment as needed.

Beagle Survey

Objectives. (1) To define the area of oceanic contamination due to fallout from the 1956 spring testing program. (2) To evaluate the radioactivity of water, plankton and fish in the contaminated areas.

If the testing program is a series of many small detonations the distribution of fallout may be so widespread that it would not be feasible to carry out an oceanic survey for the above purposes.

Area and Time The area to be surveyed would be in the vicinity of the Caroline Islands but probably somewhat northward, between Eniwajokin and Guam. Two months in the summer of 1956 (July, August) would be required. Distance traveled, from Eniwajokin to Eniwajokin, would be 5000 to 8000 miles.

Equipment. An ocean-going vessel with a low stern suitable for towing plankton nets and for hauling water bottles, and with laboratory space for processing and counting samples will be needed.

For plankton and water work a winch with towing cable and a boom or davit on or near the stern and close to the water are required. Laboratory space 150 square feet of covered area, air ventilation, and exhaust ducts for 2 counters and scalars and a drying oven are needed.

In addition, 2 counters and scalars, a drying oven, 1 ne water, 100 millipore filters, water bottles, messengers, ammeters, meter block, and miscellaneous supplies and equipment (\$500) will be required.

Operation Plan The first phase would be to outline roughly the area of concentration by sampling rapidly and at several points, beginning in the area of predicted fallout. The second phase would be a more precise measure of the distribution of fallout laterally and with depth within the fallout

area. A third phase would be to make reef collections at islands in the pattern of the contaminated water. A twenty-four-hour work schedule would be in effect while at sea.

It would be necessary to count the samples aboard ship as the sampling pattern should be adjusted to the pattern of activity of the samples.

Water and plankton samples would be taken at regular intervals. The water samples would be mostly at or near the surface with an occasional sample to 100 meters or greater. Two plankton nets would be towed simultaneously, one of coarse mesh netting and the other of fine mesh. A millipore filter would be used for filtering organisms smaller than those caught in nets.

Flying fish would be caught at night with lights and nets and sharks by hook and line. Other pelagic fish would be collected as available. The reef collections would include fish, invertebrates and plants.

Two Applied Fisheries Laboratory staff members, two assistants, and an electrician for maintaining counters and scalars will be needed.

Special Problems on Uptake of Radioactivity

The special studies on the uptake of radioactivity by various organisms would be the responsibility of a senior scientist from the Applied Fisheries Laboratory and should be arranged to coincide with the program during the period the staff is at the BML.

A. A study of the relative and total amounts of radioactive materials absorbed in organs of fishes.

This study is suggested to obtain information on the distribution of radioactive materials in the various organs of fishes as well as the total content of radioactive materials in the whole fish and the relationships involved. Previous studies (OWFL-42⁴, OWFL-43³) have indicated certain patterns in the tissues analyzed (skin, muscle, bone, liver and viscera) which seem to prevail, at least in the longer half-life materials. In these tissues the per-gram-weight relationship of radioactive materials bears the same ratio for at least a year after detonation^{3,4}. If this holds true generally for all tissues and organs, then monitoring for total radioactivity may be greatly simplified and prediction of total amounts of radioactivity in a fish may be possible on comparatively limited information. As an adjunct, information concerning the radiochemistry of certain organs might be required, and the relative contents of certain tissues and organs or systems will be obtained.

The study would, at first, be confined to the analysis of the organs and systems in a single species of a comparatively common fish. Ten or more specimens should be obtained for study during a period of one month for comparable results. The specimens should be in good condition and without injuries. The following tissues will be sampled

muscle (from about 5 body areas)	posterior kidney
skin (without scales)	air bladder
scales only	liver
circulating blood (cell and serum to be separated)	gall bladder
heart muscle	stomach wall
gill filaments	intestinal wall
brain	stomach contents
caudal vertebrae	intestinal contents
skull	spleen
opercle	pancreatic tissue
pituitary gland	eye
thyroid gland	nostril
thymus gland (?)	gonad
renal glands (?)	pyloric mass
anterior kidney	teeth
	ureter
	body fat

Approximately 30 plates per fish will result from the dissections for radioassay. In cases where only a small amount of tissue is dissected (i.e., pituitary gland, thyroid gland, etc.) the tissues from several specimens similarly treated will be pooled.

Some of the plates will be counted for decay to substantiate radiochemical analyses and studies on selective uptake of various elements. The dried tissues can be used for radiochemical analyses. In these studies the dried tissues from several specimens can be pooled and certain ones analyzed for specific purposes (i.e., thyroid and anterior kidney for iodine, bone for strontium, etc.).

It is estimated that about two fish may be dissected and analyzed or processed per day per man at the field laboratory, so that allowing for collections, approximately 10 to 15 days would be required at Inuvik for this program.

B. Proposed studies of radiostrontium levels in crustaceans

Radiostrontium has been found to constitute a large proportion (up to 50%) of the radioactivity in the exoskeleton of land crabs collected at the Pacific Proving Ground. It is proposed, therefore, to (1) compare radiostrontium levels in land crabs and marine crustaceans collected in the field, (2) make field observations of the habits of land crabs with the idea of determining their principal source or sources of radiostrontium, and (3) make determinations of rates of absorption of strontium by land crabs at the Applied Fisheries Laboratory

Collection of material for (1) and the field observations will be made at Eniwetok Atoll. Crabs for determinations of rates of absorption should be collected at Kujalein or at some other uncontaminated atoll for shipment to Seattle.

It would be most suitable to conduct these studies to days after the first series is completed or later. This would avoid confusing effects of repeated contamination with shorter-lived radioisotopes during the first series. Since ^{90}Sr has a long half life it would constitute a higher proportion of the total radioactivity at the later date, thus simplifying the study. The minimum time required would be six weeks at the 27%.

C. The absorption of radiiodine by Asparagopsis

During the 1954 Castle series a red alga, Asparagopsis, concentrated ^{131}I about 18,000 times the amount present in the surrounding water³. Other algae or land plants did not show

this affinity for I^{131} . It would be of interest to (1) make a more complete survey of the flora to determine what marine plants besides Asparagopsis absorb this isotope, and (2) determine the physiological role of iodine in the plants' metabolism. This should be done in the field because of the difficulty or impossibility of culturing Asparagopsis in the laboratory and because of the availability of the other plants being studied. Because Asparagopsis has an affinity for I^{131} , it can be used as an indicator of the time required for the radioactive particles produced by a nuclear test to reach a given area and be absorbed into the flora.

1. Survey of plants for I^{131} affinity

1. Pre-shot collections of algae would be made along with water samples at locations near the test site and behind the BML. These samples would be analyzed for total activity as well as for I^{131} activity to serve as a base line for the post-shot samples.
2. Decay studies will be included.
3. Radiochemical analyses for iodine would be made if the activity is sufficient.
4. Post-shot collections of algae and water would be made as for the pre-shot collections.
5. Decay studies will be started immediately on all post-shot samples.

2. Physiological significance of iodine in Asparagopsis

- a. Separation of pigment fractions of algae by sugar column chromatography and paper chromatography is planned.
- b. Counts of fractions for total activity and decay of some plates will be made.
- c. An attempt will be made to evaluate fractions having the major portion of the activity and to identify further the isotopes by chemical or chromatographic methods.

This study will determine where the iodine is being concentrated, whether in the pigments, water soluble compounds, or in the plant residues. The results will suggest further research on the problem of the role of iodine in algal physiology. It is thought that iodine may be incorporated in an amino acid compound making up the proteins of a proteinaceous compound, phycoerythrin, which has been found in the red algae.

Study of rat populations at Janet Island, Bakeria Atoll

Special studies with the rats (Rattus norvegicus) at Janet Island are proposed as follows.

1. Evaluation of the alteration of spermatogenesis in a population of rats exposed to several dose-range nuclear detonations and in which individual males have carried relative burdens of fission products approximating the maximum permissible concentration.

Chemical analysis of the spermatogenic wave provides an index for testis damage that is much more sensitive than direct measurement of pathological damage.

Specimens. The following specimens will be required:

(a) Ten mature male control rats from each of three different uncontaminated atolls in the Marshall or Caroline Islands. (b) Ten mature male rats from Janet Island before the device is detonated in the Mike-Nectar crater. (c) Ten mature male rats from Janet Island two weeks after the detonation of the above mentioned device. (d) Ten mature male rats from Janet Island two months after detonation.

In the event other devices are detonated near Janet Island, changes in the schedule of the program would be made to allow for the conditions imposed.

The testis should be removed from each animal at the time of capture and fixed for the study. Tissues from the rats at Janet Island should be retained for the determination of radioactive content.

The quantitative work on spermatogenesis will be done in cooperation with Dr. Edward R. Snodgrass, Department of Anatomy, University of Washington, as a joint study.

Radioactivity measurements. (a) A weekly record of accumulated β and γ radiation dose (pre-shot) should be made by means of film badges placed in holders one inch above the ground level, in the burrows, and in the instrument bunkers. (b) Initial radiation levels and total accumulated dose during the first week following detonation should be recorded as well as the levels of beta, gamma and alpha radiation at the site. These data

could be obtained from the physical measurements group. The levels of β and γ radiations at Janet should be recorded by Applied Fisheries Laboratory personnel during the period two to eight weeks following detonation.

(c) Levels of radioactivity should be determined for testis material from selected specimens taken at Janet.

2. A comparison should be made of the levels of radioactive strontium, zirconium, ruthenium, cesium, cerium, and iodine in the soil, in the food plants growing in the soil, and in the tissues of the rats eating the plants during the period two to eight weeks following detonation.

The carcasses of the rats collected for the spermatogenesis studies, in addition to those of females taken during the collecting period, would be used.

E. A proposed study of factors influencing radioactivity in sea cucumbers of the Marshall Islands

A study of the factors influencing the radioactive content of a marine invertebrate is proposed. Sea cucumbers occur in vast numbers in certain areas, approaching a concentration of one per square foot on some seaward reefs, for example, the islands north of Runit, Eriwetok Atoll, and at Enyu, Bikini Atoll. At some islands Holothuria atra, the species most widespread in the region, has not been obtainable on the circuit collections, but other species have been substituted. At Parry Island, the field headquarters, there is an unfortunate scarcity

of sea cucumbers, which would have to be compensated for by collecting in other areas. It might prove feasible to bolster the Parry population by introductions from the islands north of Anit.

The ecological role of these grazing animals, which are practically without predators, invites elucidation. Brief observation has lead to the estimate that in areas of dense population they consume the microflora and fauna from several tons of coral sand per acre per day. The contribution to the reef as a result of this conversion is not understood.

Factors to be considered should include species differences, reproductive cycles and their relation to seasons, affinities of tissues for various isotopes, and geographic distribution.

Field Facilities and Logistic Support

The Eniwetok Marine Biological Laboratory (EMBL) on Parry Island has proved to be a very fine base from which to conduct field studies such as those proposed.

The space and facilities required for the Applied Fisheries Laboratory's 1956 field program will vary during several phases of the operation. Early in the test program the major effort should be concentrated in (1) establishing the existing levels of radioactivity that remain from previous tests in the area, and (2) with the start of the tests, evaluating some of the short-lived radioactivity.

The second peak in effort should come at the termination of

the active test program, when the studies of decline in radioactivity and oceanic circulation should be emphasized.

Sharing of the facilities and space at KAMEL with the Health and Safety Laboratory group of the New York Operations Office according to the plan outlined by Dr. W. R. Boss on December 20, 1955 should allow for optimum use of the facilities.

Task Group support is needed to provide a chain of command to the various sections of the test program. Without a clear-cut directive with a high priority assigned to the program of radiation biology, the studies cannot be carried out with maximum accomplishments.

Transportation is needed to transport men and equipment about the atolls, between atolls and on the open sea.

Surface transportation by "W" boat or equivalent should be provided at Eniwetok for trips to the islands of the atoll and for making collections of plankton and pelagic fish.

Air transportation, by small plane or landing strips are available or by helicopter to other sites, should be provided on an "as needed" basis.

An ocean-going vessel with facilities for conducting plankton and water sampling at sea is needed for the suggested ocean monitoring survey (pages 8, 9, 10).

Equipment

The basic facilities at the EREL and the Applied Fisheries Laboratory will be used to the maximum. However, additional equipment will be needed for some phases of the suggested program.

The EREL should be equipped with the following additional facilities.

1. A recording counting rate meter available from

Nuclear-Chicago
223 W. Erie Street
Chicago 10, Illinois

<u>No.</u>	<u>Item</u>	<u>Est. Cost.</u>	
1	Model 21 - border	\$345.00	
2	2-36 d. n. tubes at \$50.00	100.00	
1	3-100 Astigraph	295.00	
1	1031 3 Shield	215.00	
1	1520 counting rate meter	495.00	
			\$1450.00

2. Ion tubes

5 type 100 - stainless steel,
high sensitivity, low background,
and thin window
ion tubes at \$70.00 350.00

Allen Electronic Laboratories, Inc.
1226 - 1238 Flushing Avenue
Brooklyn 6, New York

Iron shields, vertical, and count
ECL Mark 1, Model 15, or equivalent 195.00

Insulation Quarter Lab., Inc.
412 West Grove Street
Evanston Park,
Evanston, Illinois

4.	2	Waring Blenders equivalent to #58977, stirrer, electric at \$34.95	\$69.90
		Scientific Supplies Company 600 Spokane Street Seattle, Washington	
5.	5	supp for Waring Blenders #58977, sub-standard size glass containers with metal screw caps and small size blending assembly at \$6.80	\$34.00
		Scientific Supplies Company as in 4.	<hr/> \$2126.90 <hr/>

Also, additional equipment must be purchased to equip the vessel to be used in the oceanic monitoring survey (pages 8, 9, 10).

1.		Power winch, high speed, electric with 400 fathoms of cable	\$1100.00
2.	4	plankton nets, 2 each #0 and #12 at \$100.	400.00
3.	5	water sampling bottles complete at \$100.	500.00
4.	4	revere thermometers at \$50.	200.00
5.	2	Bathythermographs at \$250.	500.00
6.	1	constant voltage regulator	75.00
7.	2	Model 181 nuclear scales at \$535.	1070.00
8.	3	Anton tubes for counters at \$70.	210.00
9.	2	shields for Anton tubes at \$225.	450.00
10.		Miscellaneous laboratory supplies, fishing gear, night lights, nets, etc.	100.00
			<hr/> \$5005.00 <hr/>
		Budget for needed additional equipment	<hr/> \$1333.90 <hr/>

Personnel

The personnel needed to carry out the bio-monitoring program and the special studies during the coming test series at Eniwetok and Bikini Atolls will be drawn from the staff of the Applied Fisheries Laboratory, with the addition of specialists in the various fields as available.

To accomplish the field program with the limited personnel available it will be necessary to rotate personnel between the field laboratory and the University laboratories. This rotation must be scheduled so that the particular specialists are available in the field at the specific time when the special part of the program to which they can contribute most is active.

A tentative schedule from about March 15 to September 15 would be as follows.

	<u>March</u> <u>15</u>	<u>April</u>	<u>May</u>	<u>June</u>	<u>July</u>	<u>August</u>	<u>Sept.</u> <u>15</u>
Borchers, Raymond	X	X	X	X			
Falumbo, Ralph	X	X	X				
Olson, Paul				X	X	X	
Donaldson, Lauren	X				X	X	X
Seld, Edward			X	X	X		
Winters, Arthur				X	X	X	
Lawman, Frank					X	X	X
Reynolds, Allyn				X	X	X	
Hines, Neal*	X						

* Air Force Reserve Program

In addition to the scientific staff scheduled for work in the field laboratories, plans should be developed and put into operation to add two to four young men to the field force to act as assistants. These men might be drawn from the armed forces or from among students in biology, chemistry, physics, medicine, etc. It is believed that the greatest good would come from the inclusion of selected students on something similar to a fellowship basis to act as assistants.

Financial Support

The major cost of the field program has been covered in the 1955-1956 budget for the Applied Fisheries Laboratory. In planning the yearly program and budget, provision for a field program of about the scope of that planned for the spring of 1956 was made.

Allowance was not made in the budget, however, for the field costs of the Rongelap survey requested during October-November, 1955, nor for the special equipment needed at IHML or the oceanic plankton program.

a. Expenditures for the Rongelap resurvey of 1955

Transportation Seattle-Honolulu and return at \$250.00	\$1,000.00
Supplies	334.45
Insurance 132 days at \$1.70	224.40
Per diem and personnel expenses	<u>1,496.64</u>
Total reimbursement for Rongelap survey	\$3,055.49

b. Additional equipment for IHML 2,128.90

c. Equipment for the marine plankton program 5,005.00

\$10,189.39

References

1. Annual Report of the Applied Fisheries Laboratory, University of Washington, for the Fiscal Year 1954-1955, with Suggested Program for the next Fiscal Year. WAPL-41 (030)
2. Radiobiological Studies at Eniwetok Atoll Before and Following the Rice Shot of the November 1952 Testing Program. WAPL-33 or WF-016 (Confidential)
3. Radiobiological Resurvey of Rongelap and Ailinginae Atolls, Marshall Islands, October-November, 1955. WAPL-43
4. A radiological study of Rongelap Atoll, Marshall Islands, During 1954-1955. WAPL-42 (Confidential)
5. The Uptake of Iodine-131 by the Red Alga, Asparagopsis taxiformis. WAPL-44

Note: All of the above reports were written by the staff of the Applied Fisheries Laboratory, University of Washington, Seattle, Washington.

END