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Notes for Meeting with the Division of Biology and
Medicine on the Applied Fisheries Laboratory
Program

September 12, 1955

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I. Subjects for reports in preparation on the Castle test series

1. Summary study of the uptake of radioactive material by the plants and animals of Eniwetok Atoll based on collections during the year following the test program.

2. Distribution of radioactive materials about Eniwetok Atoll during the year following the test program. Evaluations are made on the basis of collections made at six locations around the atoll at monthly intervals.

3. Repopulation studies in the target area of Eniwetok Atoll. Detailed studies were made of the changes in reef and island ecology by the blast and the recovery during the following year.

4. Concentration and cycling of fission products in the rat populations of Engebi (Janet) Island, Eniwetok Atoll.

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5. Uptake of I^{131} by birds and rats on Eniwetok Atoll during and following the weapons tests at Bikini and Eniwetok Atolls.

6. Radiation concentration in planktonic forms in the waters of the lagoons and the sea about the Pacific Proving Grounds.

7. Uptake of radioactive materials by the tuna and other large food fish taken in the sea around the Pacific Proving Grounds.

8. Concentration of I^{131} by *Asparagopsis* on the ocean reefs at Eniwetok.

9. Study of the radiation contamination of Bikini Atoll following the weapons tests. This study is based on collections made at Bikini at quarterly intervals during the year following the weapons] tests.

10. Study of the normal radiation level of marine organisms in the contaminated areas of the northeastern Pacific.

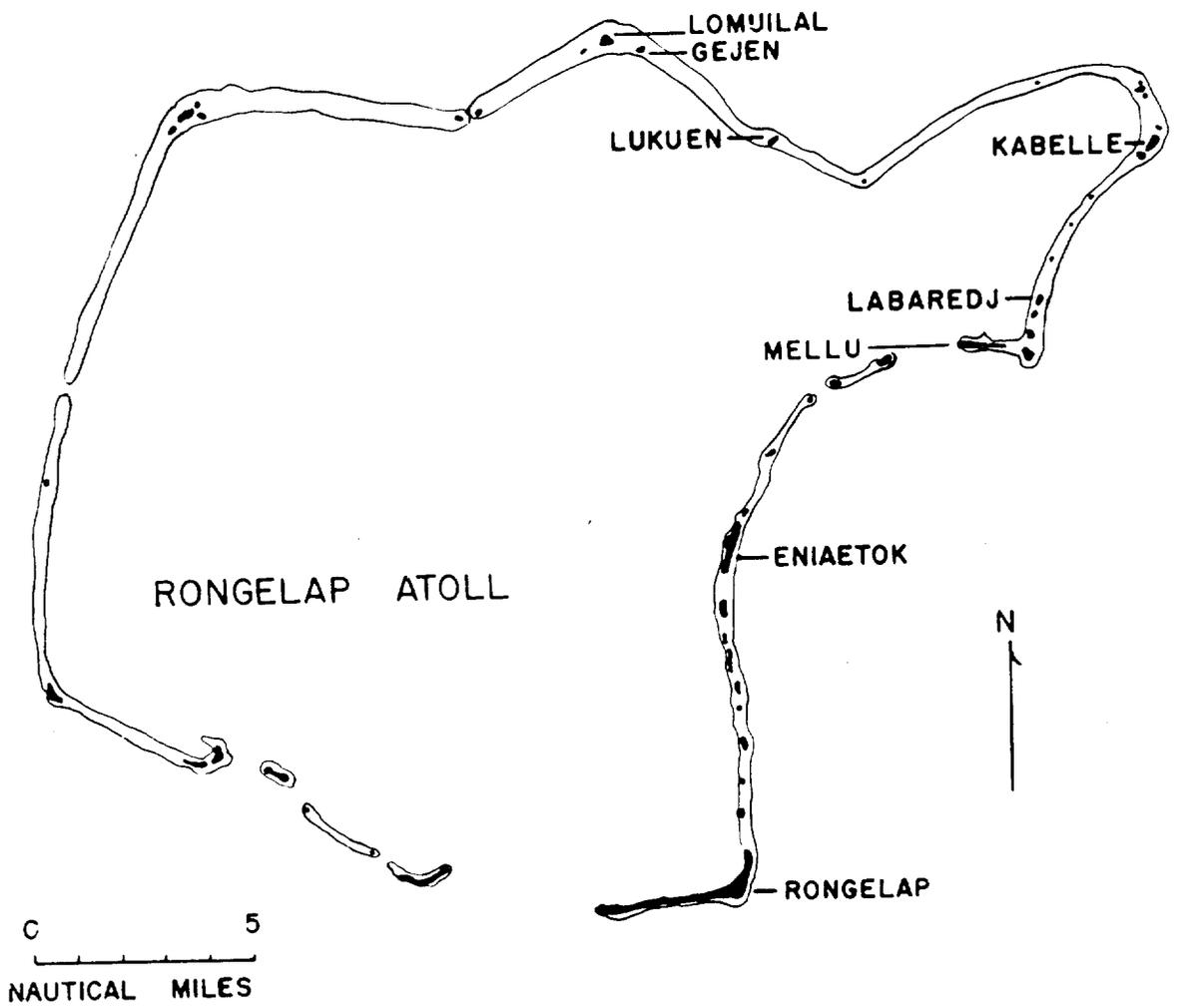


FIG.1 COLLECTING AREAS AT RONGELAP ATOLL

Table I. Radioactivity of Foods from
Rongelap Atoll, 1954-55

Values expressed in microcuries per kilogram of wet tissue

Date and Island	Coconuts Milk	Coconuts Meat	Misc. ^{1/}	Fish Muscle	Fish Liver	Clams Muscle-Mantle	Crabs Muscle	Birds Muscle	Birds Liver
3/26/54 Kabelle, Labaredj	1.03	1.16	11.3	2.74	204.	43.5	70.0	5.38	25.4
7/16/54 Kabelle	.049	.123		.423	24.0	2.14	2.39	.576	3.23
12/8 or 18/54 Kabelle, Rongelap	.019	.048	.021	.066	2.05			.040	.213
1/26-30/55 ^{2/}	.041	.036	.049	.100	3.52	1.03	.498	.129	.418

^{1/} edible portions of squash, papaya, arrowroot, pandanus, spinach
^{2/} Rongelap, Enlaetok, Labaredj, Kabelle, Gejen, Lomulal, Lukuen

Table II. Coefficient of Variation in Per Cent ^{1/}
for Values in Table I

Date	Coconuts Milk	Coconuts Meat	Misc.	Fish Muscle	Fish Liver	Clams Muscle-Mantle	Crabs Muscle	Birds Muscle	Birds Liver
3/26/54	42(4) ^{2/}	--(1)	--(1)	65(12)	119(12)	36(4)	79(3)	41(5)	38(5)
7/16/54	10(2)	13(3)		73(20)	65(20)	54(2)	35(5)	75(7)	48(7)
12/8 or 18/54	37(5)	57(5)	23(8)	48(3)	30(3)			27(4)	37(4)
1/26-30/55	61(18)	76(16)	88(16)	68(81)	97(81)	115(4)	178(11)	99(13)	95(13)

^{1/} C in % = (standard deviation ÷ mean) (100)
^{2/} number of samples

II. Summary of UWFL-42 - " A Radiological Study of Rongelap Atoll, Marshall Islands, During 1954-1955"

At Rongelap, the major portion of the fallout from the March 1, 1954 Bikini shot was over the northern part of the atoll.

It appears that radioactive material is being rapidly redistributed throughout the atoll, at least in the deeper waters. Algae collected from 10 - 25 fathoms in the south in January 1955 were higher in radioactivity content by a factor of about two than the the same species collected in the north.

In the land surveys higher levels of activity were found above high tide line than over beach sand or rock. The differences were variable with location and time after fallout; the maximum difference a month after fallout was by a factor of more than ten. Three and a half months later the maximum difference was by a factor of about four. Where the concern is with total amount of radioactive material it is clear that the reservoir of radioactivity lies at the bottom of the lagoon.

Dry land comprises less than one per cent of the total area of the atoll. In addition activity in land soil is found mainly in the top inch, while core samples have been taken from the lagoon bottom with as much activity at depths of 4 -5 inches as in the top inch. Assuming an average activity level in sand over the entire lagoon bottom of

10^4 d/m/g to a depth of 10 cm, the total activity in the lagoon bottom is estimated to be 6×10^5 curies. This is a conservative estimate based on the activity in the sand and does not include the activity in algae and other living organisms.

The phrase "rate of decline" has been coined to express the combined physical decay and the biological uptake and decay rates.

For purposes of estimating past or future levels of activity in food plants or animals where no specific information is available, the rate of decline $t^{-1.7}$ is the best approximation.

Averages of activity levels in coconuts, fish, clams, crabs and birds, all of which are used for food, are given for March, July and December 1954 and for January 1955. Of these, fish livers had the highest levels - 204 uc/kg wet tissue in March and 3.52 uc/kg wet in January, while coconut milk had the lowest levels, 1.03 uc/kg wet in March and 0.041 uc/kg wet in January.

Mixed fission products in toto are the principal radioactive contaminants of biological materials with notable exceptions such as the short half-life element I^{131} , which is concentrated by bird thyroid glands and the alga Asparagopsis. Some coconuts and crabs, by contrast, concentrated undetermined long half-life components.

The average rate of decay for 88 samples during the period March 1954 - January 1955 was $t^{-1.43}$.

Variability in levels of activity was great. Data grouped in broad categories of types of food were found to have a coefficient of variation of 10 per cent to 178 per cent. Even within single species collected at the same time and at the same location, a high degree of variability was often found. Practical considerations did not permit as adequate sampling as might be desired although these data represent the results from 1499 individual samples.

Differences between omnivorous and carnivorous fishes as to amounts of radioactivity were greatest in March 1954. By January 1955, the differences were negligible in skin, muscle and bone.

Variation in activity associated with area indicates a greater contamination of the atoll in the north than in the south during December and January. Earlier collections of fish from the south were not available so that the redistribution of activity with time, as indicated from the algae collections, cannot be evaluated from the fish data.

Activity levels in tissues were, in descending order, - viscera, liver, skin or bone, muscle. The difference between liver and muscle is commonly by a factor ^{greater} ~~of less~~ than 100.

Lagoon fish taken in the northern part of the atoll are comparable in levels of radioactivity to reef fishes taken in this region.

In the invertebrates, activity levels were highest in digestive and

excretory organs, intermediate in integumentary organs and lowest in the muscle.

Maximum differences in activity levels between species of invertebrates are by about one order of magnitude.

Both edible and non-edible plants were collected, the latter for comparison with collections from Eniwetok. The edible plants were coconut, squash, papaya, arrowroot, pandanus, spinach, and Morinda. The highest level found in January 1955 was 8.6×10^{-7} uc/^g wet weight of papaya pulp.

Activity levels are higher in the leaves than in the fruit, the difference generally being two to eightfold.

Much of the activity in the March collection was probably due to surface contamination. High counts in internal portions of stems and in leaf buds, however, indicate rapid uptake by absorption through root systems.

Both local terns and migratory shore birds were collected. Tissues of shore birds contained greater amounts of radioactive materials in March than did the terns but the rate of decline was more rapid in the former. These differences are related to differences in feeding habits and migration characteristics.

Three types of decline of activity levels in terns were found -

(a) semilogarithmic, half life 40 days - muscle, liver, kidney; (b) logarithmic, bone ($t^{-2.35}$) and ileum ($t^{-2.85}$); (c) extremely high degree of variability - skin and lung.

Contrary to expectations, in January four to six times more activity was found in the southern birds than in the northern birds. In view of this, it is suggested that the bird population of the neighboring atoll, Alinginae, be sampled for determination of activity levels. Rongelap natives collect birds at Alinginae as part of their food supply

Activity levels in eggs were low compared to other bird samples. Shell levels approximate those found in bones; yolk levels varied from one and one half to three times those found in muscle, and whites contained the lowest levels (0.009 uc/kg in December) of all bird samples.

Radioactivity of Rongelap plankton samples was more than 100 times greater than that of surrounding open ocean water samples.

Radioactivity per unit wet weight of plankton is greater than for most other biological materials.

There is considerable variation in the radioactivity of samples from paired tows.

Radioactivity in the top inch of soil (northern) was 6.8 uc/~~kg~~^g in March. The decay rate for the period March 1954 - January 1955 was $t^{-1.31}$ with March 1, 1954, as the date of origin.

For soil samples the rate of decline at Kabelle Island is greater than the decay rate from March to July, but less from July to January.

When sample counts from all islands are averaged, decline and decay rates are the same.

A conservative approximation of the radioactivity of the lagoon water in January 1955 is 0.0011 uc/l.

Fresh water samples included cistern water, filtered well water, standing water and ground water. Ground water from Kabelle Island was most radioactive, 0.022 uc/l. Radioactivity of fresh water samples increased from south to north, with the activity of Rongelap Island samples being 1/10 to 1/4 of the activity of samples from the north.

Radiochemical analyses of selected samples were made for iodine, cerium, zirconium, niobium, strontium, yttrium, ruthenium, barium, and calcium. Radiostrontium was found in plants, birds and crabs, but not in fish muscle nor in some of the coconuts. The greatest amount found in the January samples was 27×10^{-6} uc/g wet weight of coconut crab muscle.

The maximum amount of Ce^{144} found in January samples was 5×10^{-6} uc/g in crab muscle while none was found in some coconut samples.

Radiocalcium in soil samples accounted for less than one per cent of the total activity as of the date of analysis, May 1954.

Fission products in soil samples were found in approximately the expected proportions.

III. Specific projects which might be included in the next test series at Eniwetok

1. Eniwetok biomonitoring program

Since 1946 biomonitoring studies have been carried on in the Marshall Islands. This would, one might assume, have provided us with sufficient information to predict or evaluate any given situation arising from atomic testing. The entire program, however, of weapon testing continually changes, both in order of magnitude and materials used. Such changes present new and different problems each requiring detailed study.

During the forthcoming series it is proposed that the biomonitoring program be continued, but reduced to the absolute minimum so as to make possible a greater concentration on other projects.

2. Study of the distribution of radioactive materials in the organs and tissues of fish

(1) Heretofore only five to seven tissues - skin, muscle, bone, liver, viscera, gonads and gills - from the fish have been assayed for radioactivity.

(2) Recent investigations indicate that different parts of the muscular system and alimentary tract may vary considerably in the amounts of radioactive materials present at any one time.

(3) It is proposed that a detailed analysis of all organs and tissues be made, preferably of large fishes, to determine the relative amounts of radioactive materials present and to identify the specific elements involved.

(4) The assay should be coupled with a detailed chemical and radio-chemical analysis of the tissues so that basic knowledge can be obtained concerning the cycling of radioactive materials in fishes.

3. Monitoring program for the determination of the possible radiation contamination of oceanic fishes of the western Pacific

The tuna fishing industry on both sides of the Pacific is extremely concerned about possible effects upon their markets from another "hot tuna" scare.

A broad monitoring program would provide industry and the Commission with the information essential for evaluating any situation that might arise.

Monitoring the fishes at sea presents too great a problem. It is proposed instead that the commercial catch, especially of the Japanese and Marshallese fishermen, be used for obtaining the samples for radiation determination.

The Japanese tuna fleet operates over the entire western half of

the central Pacific. The boats, however, return with their catch to three ports in Japan. At these ports the fish are sampled by drilling a very small core for organoleptic testing. A portion of the core, especially of the part used for food, could be obtained, dried and mailed to the Laboratory for evaluation.

At Ponape, Caroline Islands, a small commercial fishery is operated by native fishermen who market their catch in Guam. This fishery affords another source of samples readily available with little or no expense.

4. Ponape, Caroline Islands, as a laboratory for studying wash down of radioactive materials from a land mass

UWFL-40 (Radiation Levels in Biological Samples Collected at Ponape, Caroline Islands, December 16-17, 1954) presents the results of an exploratory study conducted at Ponape in December 1954. Even at the late date of our initial investigation, the relationship of the collecting basin to the adjacent water body seemed evident.

During the next test series as a second interest to the monitoring of tuna fish at Ponape, a very useful block of information on the wash down of radioactive fallout into a harbor area may be available.

The atolls of the Marshalls and the Nevada Proving Grounds do not have the combination of land mass, heavy rainfall, and harbor area to carry out such a study. Ponape, however, is ideal for this type of study, as facilities are available for carrying out the study, and the island is frequently in the path of fallout from weapons tested at Bikini or Eniwetok.

5. Plankton

The "Taney" plankton samples have been processed, counted and the data analyzed. The report of these data has been forwarded to NYOO. The Rongelap plankton data are reported in UWFL-42. The Eniwetok, Bikini and other Marshall Islands plankton samples have been processed, counted and the findings are now being tabulated.

As a follow-up on the "Taney" collections it would be advisable to determine the amount of radioactivity in plankton samples now being collected in the north Pacific by operation Nor Pac. The pattern of distribution of radioactivity found from both the "Taney" plankton and from water samples suggests a possible northward extension beyond the limits of the "Taney's" operation. The area and timing of the plankton collections by Nor Pac are right to offer more positive information on

this assumption. In addition, background information on radioactivity of oceanic plankton could be obtained.

If the samples are available, collections from the Hawaiian Islands northward would be desirable. Plankton collections by the Oceanography Department of the University of Washington will be worked up at the University. Availability of this material is subject to the consent of Drs. Fleming and Frolander. The amount of material desired is about one gram, wet weight, per sample.

A total of 500 samples could be processed by the Applied Fisheries Laboratory without additional help. If the number of samples was much greater a technical assistant would be required. If samples are available from all areas of the Nor Pac operation then more than 500 samples may be desirable. We understand that some request already has been made by the AEC or an AEC organization for similar samples and we should like to know the nature of this request and what is being done.

6. Rats

There is a colony of field rats (Rattus exulans) on Janet Island in the northern part of Eniwetok Atoll. This population has been exposed to lethal and sublethal doses of gamma and neutron radiation four times

during the interval of 1949-1954. After short periods of decimation, subsequent to the various exposures, the population has exhibited a surprising ability to recover from the damage and to increase greatly in numbers. In general, the members of the colony are in excellent physical condition, and when indices of radiation damage such as lethal mutations, abnormal embryonic development, and reduction of fecundity are applied, they are found to be no different from those found in non-irradiated populations of the same species. Although the bones of these rats, as of January 1955, contained Sr⁸⁹⁻⁹⁰ in amounts approximating the maximum permissible dose, bone tumors have not been found.

The above described colony is of special interest concerning the ability of a population of animals to survive and multiply in the presence of supposedly lethal initial doses of radiation and subsequent heavy body burdens of radioactive isotopes. In view of this fact an outline of a proposal for a continued study of the population was forwarded to the Division of Biology and Medicine, USAEC in June 1955.

An outline of the proposal is as follows:

I. Life history

A. Habitat

1. Physical characters (mapping of the area, determination of amounts of radioactivity and identity of the isotopes in soil of the area

2. Food supply (amount of food plant growth/year/unit area, specific activity and isotopes in food, seasonal changes, list of food items, amount of food eaten/animal/unit time, food conversion)
3. Burrows and nests (enumeration and description)
4. Patterns of runs
5. Endoparasites and disease

B. Characteristics of the rats

1. General behavior
2. Reproduction (average gestation period, prevalence of pregnancy, incidence of pregnancy, embryos/litter, number young/year, sex ratios in young, embryo size at birth, survival index of ova, sperm counts, size at maturity, examination of testes for abnormal spermiogenesis and spermatogenesis. *
3. Mortality (total death rate, sex differences, average age, seasonal variation)
4. Population characteristics other than reproduction and mortality (total number, distribution, rate of increase, movement)

II. Studies of species characters (taxonomic)

III. Radiation effects on individual rats

- A. Pre-shot studies (100 rats). (Examinations for lesions,

* To be done by Dr. Roosen Runge, Dept. of Anat., Univ. of Washington

blood counts (RBC, WBC, differential, Hb, hematocrit, platelet), urine sugar and albumin, examine embryos for abnormalities, ability to survive under confinement, weight, radioisotope content and excretion

- B. Post shot studies (200 rats). (Six stations to be placed at given distances from ground zero prior to shot, 30 rats/station, protect from heat and blast, recover at identical times, 11 rats/station for histological and radiochemical analysis, 22 rats/station for studies listed under pre-shot studies, III A)

Genetic studies with this group of rats would pose many problems which could not be overcome in the year allotted to the experiment. If genetic studies are desired they should be done with animals of which the genetic history is known. Investigations of this type have been done in great detail by other workers.

7. The absorption of I^{131} by Asparagopsis

In the 1954 Castle series it was shown that a red alga, Asparagopsis, concentrated I^{131} about 18,000 times the amount present in the surrounding water; other algae or land plants did not show this affinity for iodine.

It would be of interest to (1) make a more complete survey of the flora to determine what plants besides Asparagopsis absorb this isotope, and (2) determine the physiological role of iodine in the plants' metabolism. This can be done better in the field because of the difficult or impossible culture of Asparagopsis in the laboratory and because of the availability of the other plants being considered. 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 biota of this area.

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

- A. Pre-shot collections of algae and land plants would be made along with soil and water samples at locations near the test site and behind the laboratory (EMBL). These samples would be assayed for total activity as well as for I^{131} activity to establish a base line for the post shot samples.
- B. Decay and mass absorption studies would be included wherever feasible.
- C. Chemical analyses for I^{131} would be made if activity is sufficient.
- D. Post shot collections of algae, land plants, soil and water would be made as for above.
- E. Decay studies would be started immediately on all samples.

F. Mass absorption curves would be made immediately.

II. Physiological significance of I^{131} in Asparagopsis

- A. Pre-shot collections of the algae at several spots to determine activity
- B. Separation of pigment fractions of the alga by sugar column chromatography and paper chromatography
- C. Counts of fractions for total activity I^{131}
- D. Evaluation of fractions having the major portion of the activity, and its further identification by chemical or chromatographic methods

This study will determine where the iodine is being concentrated, whether this is in the pigments, water soluble compounds, or in the plant residue. The results will orient further research on 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, phycobilin, which has been found in the red algae.

The use of Asparagopsis as an indicator of radioactive fallout and recycling would require that collections be made at various locations before a shot and periodically afterwards. The amount of I^{131} in the samples would determine the time required for the radioactivity produced by the most recent test to reach a given area and be absorbed by the alga. Later collections would enable one to determine whether recycling of the radioactive products had taken place.

8. Recycling of fission product material in the lagoon at Eniwetok as determined by radioactivity studies on algae, coral, soil and water samples

Observations made during the Castle series indicate that a large portion of the radioactivity resides in the deeper waters of the lagoon and that there is a possibility this radioactivity is being recirculated from the north end of the atoll to the south end. Preliminary data is insufficient to establish with certainty the pattern that exists. It is felt that an elaboration of our 1954 program, in this respect, would clarify this problem. It would enable us to determine the amount of radioactivity in the biological and physical components of an area which has received little attention in previous experiments. The mass of water and sand in the lagoon is a storehouse of radioactivity which is continually given off and absorbed by living organisms.

Rate-meter counts of the samples collected would give indications of activity and requirements for further collections. These counts would be combined with more accurate counts made at the laboratory with more sensitive equipment.

Chemical analyses of the most active fractions, with energy and decay measurements, would help to determine the isotopes present.

We know little about the form in which this radioactivity is present. Is it in ionic form, or is it adsorbed on silt particles or on planktonic forms? The particle size containing the major portions of the activity could be determined.

The collecting program for this experiment would follow these

lines:

	<u>Samples</u>	<u>Islands</u>	<u>Time of Collections</u>
Soil	a) island	1) as close to X as possible	a) immediately before X
	b) intertidal	2) Alice - Belle	b) immediately after X
	c) 1 mile offshore in lagoon	3) Leroy	c) periodically after X (monthly or longer)
Algae	a) <u>Halimeda</u>	4) Glenn - Henry	
	b) <u>Caulerpa</u>	5) Elmer	
	c) <u>Asparagopsis</u>	6) Jieroru	
Coral	a) Acropora	7) Bruce	
Water	a) deep water	8) Yvonne	
	b) shallow water	9) Vera	
		10) Olive	
		11) Janet	
		12) Mack	

Water samples - counts to be made:

- 1) non-filtered
- 2) filtrate
- 3) residue
- 4) K^{40} with flame spectrophotometer

Number of samples required per collection at each island:

(triplicate samples)

algae	$3 \times 2 = 6$
island soil	$3 \times 1 = 3$
intertidal soil	$3 \times 1 = 3$
deep-water soil	$3 \times 1 = 3$
coral	$3 \times 1 = 3$
shallow water	$3 \times 1 = 3$

total 21 samples per island per collection

21 samples / island

x 12 islands (this may be reduced considerably if desired)

total 252 samples per collection around the atoll

at least 3 collections should be made: $252 \times 3 = 756$ samples total

Requirements at the Eniwetok laboratory:

1. 2 - 3 men with aqualung experience
2. 3 aqualungs with spare tanks
3. M-boat support
4. rubber life raft from which to operate during diving operations
5. 1000 vials and containers for samples
6. rate meter
7. 2 Geiger counters
8. buoys and anchors to make collecting stations - 3 at each island
31 buoys and anchors
9. laboratory apparatus - mostly available now at EMBL
(require additional funnels, filter paper, etc.)

IV. Studies on the productive capacity of water areas and the methods for increasing production

During the past year work has continued in this field on a number of projects.

The development of stocks of fish with the capacity for growth, survival in warmer waters, increased fecundity, resistance to disease, better conversion of food, etc. has shown marked progress.

Studies with interracial hybrids of cutthroat trout produced six times as many fish as did the original stocks.

The Washington State Department of Game has assigned Echo Lake, a 13.1-acre body of water, for our use in productivity studies. The physical and biological studies on Echo Lake have progressed to a stage where we can now begin to carry out the specific projects designed to increase productivity.

Comments: