UPTAKE AND ELIMINATION OF ^{137}Cs IN AQUATIC INSECTS

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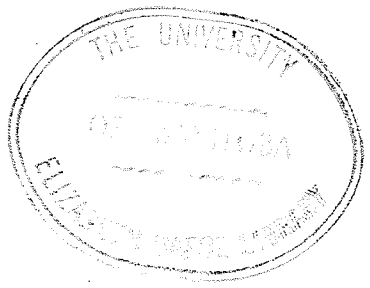

by

John Erskine Guthrie

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ABSTRACT

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Some applications of ^{137}Cs as a radioactive label for entomological investigations in the laboratory and in natural situations are described.

Accumulation and elimination of ^{137}Cs by an aquatic Hemipteran Lethocerus americanus, the giant water bug, has been studied. Instar IV and adult water bugs eliminated ^{137}Cs at different rates. The rate of labeled-prey consumption required to maintain the insect's steady-state level of ^{137}Cs was calculated. It is shown that knowledge of an organism's method of feeding is essential to successful use of the radioactive label technique of estimating food consumption.

A study of ^{137}Cs uptake by two species of mosquito larvae (Aedes aegypti and A. atropalpus) showed accumulation of the radionuclide was suppressed by the concentration of potassium in the larval rearing medium. It is postulated that most of the ^{137}Cs entered the larvae by diffusion across the papillary membrane, and not via the gut. Consequently, ^{137}Cs is not considered to be a suitable radionuclide for use as a label to measure food consumption by mosquito larvae. It readily leaches out of the food into the rearing medium.

A 19 m diameter permanent pond was contaminated with 0.5 Ci of ^{137}Cs, most of which was sorbed onto the pond mud. The integrated dose of

ionizing radiation delivered to the pond's benthic organisms was greater than 2 rad/day in some locations. A simple method for measuring the distribution of the radiation dose in the pond employing lithium fluoride dosimeters, was developed. Algal growths concentrated most of the ^{137}Cs taken up by the pond biota. Chironomid larvae had the highest specific activity of all the pond insects which were measured.

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Last and not least, I gratefully acknowledge my gratitude to my wife and daughters for their encouragement, and their constant cheerfulness in the face of not infrequent bouts of bad temper.

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CHAPTER I

INTRODUCTION

Radionuclides are radioactive species of stable elements. A characteristic property of any radionuclide or radioactive isotope, is that it decays. Radioactive decay is the process whereby radionuclides undergo spontaneous disintegration during which energy is liberated, generally resulting in the formation of new nuclides. This process is accompanied by the emission of one or more types of ionizing radiation such as alpha or beta particles, and gamma photons. Ionizing radiation (radiation) is any electromagnetic or particulate radiation capable of producing ions, directly or indirectly, in its passage through matter. The radiation emitted by radionuclides can be detected and measured with a variety of instruments, and it is this property which makes them a unique experimental tool.

The experimental use of radionuclides pre-dates the explosion of the first atomic device in 1945. Shortly after the discovery of X-rays, Malding and Thouvenin (1898) reported their effect on seed germination. By 1904, a biological application of autoradiography, the production of a two- or three-dimensional image on a photographic plate by radiation, had been reported by London (1904). The biological uses of radionuclides has been discussed in historical perspective by Hevesy (1948, 1951), a pioneer in tracer applications. Since 1945 the use of radioactive isotopes and radiation in general biology and entomological studies has become established. These uses are the subject matter of

text-books by Kamen (1951), and O'Brien and Wolfe (1964), for example.

For more than 50 years X-rays have been the major source of acute radiation used by workers studying biological effects on cells, tissues and organisms (Bacq and Alexander, 1961). The cost of running X-ray equipment continuously, however, precludes its use as a continuous-radiation source. The study of the ecological effects of chronic exposure to radiation was delayed until radionuclides were obtainable in sufficient quantities to make multi-curie sources. The use of such sources for the irradiation of terrestrial habitats, and the observed effects of these exposures have been the subject of numerous studies in the past ten to fifteen years. Many of these have been part of major gamma irradiation projects such as the Brookhaven Irradiated Forest (Woodwell, 1963; Bower, 1965), the Nevada Test Site Irradiator (French, 1964), and the Puerto Rico Rain Forest Irradiation Project (Odum, 1964). Interest in the ecological effects of nuclear warfare stimulated the development of most of these large dose-rate investigations (Woodwell and Sparrow, 1965). Studies devoted to the ecological effects of radiation in aquatic habitats are not as numerous as those of terrestrial environments, particularly at low dose-rates.

The uptake and elimination of ^{137}Cs by the giant water bug (Lethocerus americanus) and two species of mosquito, the distribution of ^{137}Cs in a pond which was contaminated with this nuclide and the integrated radiation dose which the pond's benthic organisms received, are the subject of this dissertation.

^{137}Cs as an Ecological Tracer

The use of radionuclides to study biogeochemical cycling and trophic level kinetics, originated with concern about the fate of radioactive wastes entering food-chains in which man was a consumer (Odum, 1959). Nuclear weapon debris continues to be the major source of man-made radioactive environmental contamination, while the contribution from discharge of radioactive wastes is small (United Nations, 1962). The biogeochemical cycling of nuclides in aquatic and terrestrial ecosystems is important because the amounts accumulated as a result of physical, chemical and biological concentration processes (Guthrie, 1962; Davis et al., 1963), determines the magnitude of the internal radiation dose received by organisms. Many radionuclides are translocated in ecosystems, thus the ecologist is provided with a unique tool for studying animal dispersion, foodweb relationships, and productivity (Schultz and Klement, 1963; Odum, 1959, and Olson, 1965). Radionuclides may be used to measure feeding and food assimilation rates in two ways: 1) The amount of tracer used may be small relative to the equilibrium body burden of the animal, or 2) the body burden may be increased to achieve a new steady-state level (Southwood, 1966). The latter method was first described by Davis and Foster (1958), and was developed and applied by Crossley and Pryor (1960), Crossley and Howden (1961), and Crossley (1966), who used ^{137}Cs.

Radiocesium (^{137}Cs) is distributed throughout the animal body after ingestion. The physical half-life of ^{137}Cs is about 30 years (Katcoff, 1960). Its biological half-life depends on the animal and the temperature of its environment (Davis, 1963). Radiocesium is suitable

for application to food consumption studies (Crossley, 1963), especially since its measurement does not require that the experimental animal be killed. However, with the exception of the work of Pendleton and his co-workers (Pendleton, 1957, 1960, 1962, and Pendleton and Hanson, 1958) who investigated the trophic level relationships in aquatic communities, no use has been made of ^{137}Cs to investigate the feeding habits of aquatic insects.

Radiocesium is of importance for another reason - its presence in fission products. Of all the fission products which are potential long-term contamination hazards to the environment, strontium-90 and cesium-137 constitute the greatest threat. The yield of ^{137}Cs from uranium-235 undergoing fission by thermal neutrons is about 6% of the total fission product inventory (Katcoff, 1960). Consequently ^{137}Cs is abundant in old fission products, and can be expected to comprise a significant percentage of delayed fallout. (Delayed, or global, fallout is that which comes down on an area remote from the site where a nuclear device has been detonated at or near ground level). Of all the sources of radioactive wastes listed by Neal (1960), the solutions from primary separation plants processing nuclear fuel bundles contain the largest amounts of fission products. Cesium-137 constitutes a significant fraction of these fission products. It has been calculated (McCullough, 1957) that irradiated (spent) fuel elements from a nuclear reactor, producing one megawatt of power will contain about 10^5 Curies (Ci) of fission products. This calculation is based on 100 days of irradiation, followed by 100 days cooling. The highly radioactive wastes from fuel processing plants are not discharged to the environment.

The emphasis of the Canadian nuclear power program is on heavy water moderated, natural uranium fuelled, liquid cooled reactors (Nuclear News, 1968). Should the primary coolant escape, the greatest potential threat would be its effect on the aquatic environment. It follows that knowledge of the fate of radiocesium and other fission products in lakes and rivers, is important to those in Canada concerned with the surveillance of radioactivity in the environment (Guthrie, 1963, 1964; Samuels, 1966a and b).

CHAPTER II

MATERIALS AND METHODS

The Experimental Ponds

In October, 1966, two ponds were dug by a small bulldozer in a strip of land along the edge of a stand of young aspen (Populus tremuloides) and balsam poplar (P. balsamifera). The experimental plot was once cultivated farmland, which was left to return to its native vegetation when purchased by Atomic Energy of Canada Limited. This plot is now one of the study-sites within the controlled, or restricted, area of the Whiteshall Nuclear Research Establishment (W.N.R.E.). The average height of the tree stand is 3.5-4 m, and the ponds are situated 35 m apart on the south side of this stand. A view of the radioactive pond is given in Figure 1, and Figure 2 shows the surrounding area. Each pond was lined with 5 mil black plastic sheeting, to reduce water loss. The sheeting was covered with 15 cms of the excavated soil. The completed ponds measured about 19 m in diameter and sloped uniformly down to the center to a depth of 2 m. After the spring thaw in 1967, both ponds were full of water as a result of melting snow and spring rainfall. The depth contours of the radioactive pond, measured in June 1967, are shown in Appendix 'A'. No plant or animal life was artificially introduced into either pond. The communities developed by invasion from the surrounding terrain, or by chance introduction of renewal buds or other reproductive bodies with the excavated earth used to cover the plastic sheeting.

In May 1967, 0.5 Ci of ^{137}Cs chloride solution was added to one

Figure 1

The radioactive pond as it appeared in midsummer 1968.

Figure 2

Aerial photograph of the experimental ponds showing surrounding terrain. The radioactive pond appears on the lower left side of the photograph, and the non-radioactive pond is to its immediate right.

of the ponds, subsequently called the RADIOACTIVE POND. After mixing, replicate samples of pond water were taken from several locations on the radioactive pond for radiochemical analysis of ^{137}Cs, to determine the uniformity of the nuclide's dispersion. The other pond was called the NON-RADIOACTIVE POND. The level of radiocesium in this pond was about one hundred times less than the amount in the radioactive pond and is attributed to fallout.

Sampling

A grid system was established at each pond to orientate and record sampling of the bottom-mud and benthic organisms. A row of wood-stakes, spaced 1 m apart, was placed on opposite sides of each pond. Pre-stretched, 50 mm OD, polyethylene ropes marked off at 1 m intervals provided a simple but effective 1 m^2 grid when stretched across the pond between stakes. To facilitate grid-square identification, the stakes were lettered 'A' to 'Z' and the rope markes numbered 1 to 22 (Appendix 'B'). The rows of stakes were laid out using a transit. A pre-determined mark on each rope was used to gauge the amount of tension applied when fastening it to the stake.

Ten samples were taken from the bottom of each pond at monthly intervals during the period May to October, 1967 and 1968. No grid-square was sampled more than once, and sampling locations were selected by a computer program which generated randomized sets of coordinates. A bottom-sampler, developed by Ireland (1968a), was used to take mud samples measuring 0.029 m^2 and 1 cm in thickness. Free swimming

(nektonic) insects were sampled by dip-net. The mud samples were taken to the laboratory for examination on the sampling day. Each sample was spread out on a white enamel tray and the insects removed with forceps. The insects were freed from adhering debris by floating them in a saturated sodium chloride solution. They were then rinsed in distilled water and stored in vials of preservative (70:5:25 ethanol: glycerol: distilled-water). A portion of each mud sample was retained for total-beta counting. This is a gross beta counting procedure, compared to counting a radio-chemically pure nuclide, because all beta particles regardless of origin are counted. The implicit assumptions of the method including the use of ^{40}K as an equivalent standard, have been described by Guthrie and Grummitt (1963). Whenever total-beta counting was employed, ^{137}Cs was the dominant beta-emitting nuclide present.

Study Species

Lethocerus americanus (Leidy), Belostomatidae:Hemiptera (Figure 3), was chosen as the species of aquatic hemipteran to be studied. Hemipterans were of interest for two reasons. First, they commonly feed from more than one trophic level. This is especially true of the giant water bug L. americanus. Secondly, they do not completely ingest their food. The piercing-sucking mouthparts are used to draw out the body contents of the prey. To date, no attempt has been made using the radiotracer method to estimate food consumption of insects which feed in this manner. The measurement of food consumption

in the field, by insects which feed by sucking their prey, is difficult. The radionuclide technique appeared to offer a practical solution.

Two mosquito species Aedes aegypti (L.) and A. atropalpus (Coquillett) were chosen as examples of aquatic Diptera to which the tracer method could be applied. An equally important reason for studying Dipterans resulted from the suggestion by Peredel'skii and Bogatyrev (1959a and b) that aquatic insects emerging from habitats contaminated with fission products, could spread them throughout the surrounding terrain.

Principle of Feeding-rate Determination Using an Isotope

The technique of food consumption estimation using radioactive tracers has been described by several workers (Crossley, 1963, Crossley and Howden, 1961, and Kevern, 1966, for example). Southwood (1966) and Reichle (1967), and Schlagbauer (1967), have reviewed the pertinent principles. Insects may consume radionuclide-labelled food at a constant rate, but its elimination occurs at a rate proportional to the amount in the body -- the body burden (Crossley, 1966, and Southwood, 1966). At steady-state, the tracer will be lost at a rate ($\lambda . Q_s$,) where λ is the biological elimination rate constant and Q_s is the steady-state body burden. The steady-state condition is maintained only if the rate at which the tracer is eliminated by the insect is balanced by the amount which is ingested (I.F), where I is the quantity of food ingested and F is the fraction

of ingested food that is assimilated. Thus we may write:

$$I.F = \lambda . Q_s$$

The elimination rate is usually written in terms of the biological half-life (T_b). By definition, T_b is the time required for the organism to eliminate one-half of its body burden of a given nuclide.

Let the body burden at time t be Q_t.

The rate of change of the body burden with time is described by the differential equation:

$$\frac{dQ_t}{dt} = -\lambda . Q_t$$

Re-arranging:

$$\frac{dQ_t}{Q_t} = -\lambda . dt$$

$$\therefore \int \frac{dQ_t}{Q_t} = -\lambda \int dt + C$$

$$\ln Q_t = (C - \lambda . t)$$

$$\therefore Q_t = e^{(C - \lambda . t)} = e^C . e^{-\lambda . t}, \text{ or } Q_t = Q_o e^{-\lambda . t}$$

The instant that feeding of labelled food is discontinued, $(t = 0)$, $Q_o = Q_s$. At any subsequent time, therefore, $Q_t = Q_s . e^{-\lambda . t}$.

When $t = T_b$, $Q_t = \frac{Q_s}{2}$,

$0.5 = e^{-\lambda . T_b}$,

$\ln 0.5 = -\lambda . T_b$

$\therefore T_b = \frac{\ln 0.5}{\lambda} = \frac{0.693}{\lambda}$

Determination of ^{137}Cs Uptake and Elimination

A small laboratory colony of Lethocerus americanus was established by hatching water bug egg clusters obtained from the non-radioactive pond. The cannibalistic character of the nymphs and adults of this species necessitated individual rearing. The rearing procedure followed was similar to that described by Rankin (1935). Each egg cluster was supported on a block of wood (5cm x 5cm x 0.5cm) floating freely on water contained in refrigerator trays (30x19x10cm). As the eggs hatched the nymphs crawled off the block and entered the water. Soon after hatching, the nymphs were transferred to individual plastic food-freezer boxes, measuring 10x10x8cm, closed with a perforated

plastic lid. A small block of wood was placed in each box, to give the nymph an object to cling to. As observed by Rankin (1935), tadpoles were found to be the most satisfactory food source on which to rear L. americanus in captivity. The accumulation of radiocesium by the water bugs was determined by feeding the insects ^{137}Cs labeled tadpoles collected from the radioactive pond. The elimination rate of the nuclide was measured after the water bugs had reached the steady-state, by feeding non-radioactive tadpoles obtained from the non-radioactive pond. The tadpoles collected from the non-radioactive pond also contained some radionuclides as a result of ingesting the fission products entering the pond via fallout. However the level of ^{137}Cs was found to be less 1/1000 of that in the tadpoles taken from the radioactive pond. Radioactive and non-radioactive pond tadpoles were collected daily with a dip-net. The water bugs were fed once per day, immediately after measuring their radiocesium content. Dead tadpoles were removed from the plastic boxes soon after the insects finished feeding. Otherwise, decomposing tadpoles would have polluted the water sufficiently to kill the water bugs (Rankin, 1935).

An experimental colony of Aedes aegypti and A. atropalpus was established by hatching eggs supplied by Dr. R.A. Brust, Department of Entomology, The University of Manitoba, and with the exception of food, his rearing methods were followed throughout (Brust, 1968). The hatching medium was a suspension of 100 mg of nutrient broth[1] in 100 ml

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[1] A product of Difco Laboratories, Detroit, Michigan.

of distilled water. Mosquito eggs were placed in 25 ml vials filled to capacity with hatching medium. A. aegypti eggs were left in the hatching solution for two hours, and A. atropalpus eggs for 24 hours. Groups of 100 first instar larvae were transferred to opaque white, plastic rearing pans (30x19x10cm) fitted with a lid. A. aegypti were fed ^{137}Cs labeled or un-labeled ground Gaine's dog meal. A. atropalpus larvae were fed labeled or un-labeled fish rearing food[1]. The larvae were not allowed to deplete the food. Since there was superfluous food, new media was usually supplied on alternate days to prevent growth of harmful bacteria. About 50 mg of powdered live yeast was added to each tray with the first feeding. The water level in the rearing pans was such that the larvae could breathe and eat at the same time. The larvae used in the radiocesium uptake experiments were given a change of medium, daily.

Measurement of ^{137}Cs

The concentration of radiocesium in the various specimens and samples, including mosquito larvae and water bugs, was measured by low level beta counting or by gamma spectroscopy. Cesium-137 emits 0.52 MeV beta particles and its daughter product emits a 0.662 MeV gamma photon, the result of internal transition to ground state. The principles and applications of beta counting and gamma spectroscopy have been described in several texts, for example those of Cook and Duncan (1952), and Gatrousis and Crouthamel (1960).

Total beta counting was the method used to measure ^{137}Cs in

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[1]Tetra Min baby fish food "E" and "L" mixed in equal amounts. A product of Tetra Kraft Werke Dr. rer. nat. Baensch Melle, Western Germany.

all insect specimens, other than L. americanus, and tadpoles. With the exception of L. americanus, and juvenile and adult mosquitoes, specimens were blotted dry, individually weighed, then dissolved in a minimum amount of concentrated nitric acid. After digesting in a hot water bath, the solutions were transferred to tared stainless steel counting trays and evaporated to dryness under infra-red lamps. The residue on the counting trays was covered with a 1:10 solution of collodoin in acetone, dried, then counted. The collodoin layer bound residue particles together on the tray which otherwise might have been jarred loose, contaminating the counter. A Sharpe's Low Background Beta Counter, equipped with an automatic sample changer and a gas-flow end window detector, was used for all beta counting. The background measured by this equipment with a stainless steel counting tray under the detector was 0.4 ± 0.05 standard deviation (SD) counts per minute (CPM). The ^{137}Cs counting efficiency of the detector, determined by counting standard sources of comparable self-absorption and geometry, was 42%.

The change in counting rate attributable to ^{137}Cs uptake was the parameter of interest when measuring radiocesium accumulation. Therefore relative counting under conditions of comparable geometry, sufficed. Individual mosquito larva or adults were fastened into a shallow depression in the center of a copper counting tray with the collodoin mixture and the beta emission counted. The average background measured with these trays in place under the detector was 0.50 ± 0.10 cpm.

The radiocesium level in L. americanus nymphs and adults and other large organisms such as frogs and small mammals, was measured by a gamma spectrometer. The spectrometer system consisted of a 7.6cm x 7.6cm NaI (Tℓ) scintillation crystal detector housed in a Heath-type shield made of 15 cm thick steel and lined with OFC-grade copper sheet (Heath, 1964). Individual water bugs were counted by placing them in small plastic pouches made from thin plastic film. The pouch containing the insect was gently taped on top of the scintillation crystal, and counted for 10 min. The insect was weighed immediately after counting, then fed. The net counting rate was determined by subtracting the insect's background count-rate from the gross counting rate. Background counting rates were determined for each insect before feeding radioactive tadpoles. As rate of change of counting was the parameter of interest, it was not necessary to determine counting efficiency of the detector. The energy calibration of the spectrometer was checked daily using a ^{137}Cs standard source. The radiocesium activity in each water bug in counts per minute was obtained after subtracting the applicable background, by integrating the area under the 0.662 MeV photopeak.

CHAPTER III

THE GIANT WATER BUGS

Introduction

The giant water bug Lethocerus americanus (Leidy): Belostomatidae, was chosen to study the uptake of ^{137}Cs by an indigenous species, and to investigate application of the radioactive tracer technique to determine food consumption by an insect with piercing-sucking mouthparts. A brief resume of the life history of this species is of interest. Bionomic information of L. americanus from Pinawa, Manitoba, is included here.

L. americanus is a predaceous insect, which captures and sucks out the contents of a variety of prey including aquatic insects, tadpoles, and small fish (Brooks and Kelton, 1967). L. americanus at Pinawa has been observed to capture and feed on large, adult, predaceous diving beetles (Dytiscus sp.). The capture of small frogs living in the radioactive and non-radioactive ponds has also been observed. This insect's "bite" is also reported to be quite painful to humans (Batchley, 1926; Brooks and Kelton, 1967). Cummings (1933) commented that the giant water bugs have attracted more attention than any other aquatic Hemipteran. Observations on the insect's occurrence and life history have been given by Torre-Bueno (1906), Hungerford (1919, 1925), Hoffman (1924), Batchley (1926), Cummings (1933), and Rankin (1935). The giant water bugs have been called "fish killers" and are widely known in North America under the

name "electric-light bugs" (Cummings, 1933). Most members of the family Belostomatidae are large (40 to 110 mm in length), flat, brown insects, with hind legs ciliated and flattened for swimming (Figure 3). They have short, flat, strap-like, retractile appendages at the tip of the abdomen. Their antennae are concealed in pockets underneath the eyes, and the prominent raptorial forelegs are a distinguishing feature. Near Pinawa, Manitoba, local ponds and quiet pools in streams are typical habitats of L. americanus, and they have also been seen near the banks of the Winnipeg River.

Occurrence

According to Brooks and Kelton (1967), the Belostomatidae appear to have no close relatives, but are usually placed near the Nepidae in phylogenetic diagrams. The family is divided into two sub-families, comprising four genera. Most species are tropical, however, 20 species are recorded from North America (Brooks and Kelton, 1967). According to these authors, species of the genus Belostoma and Lethocerus are widely distributed in North America. The genus Benacus is confined to the eastern United States and Canada. Abedus is primarily a western genus.

The occurrence of three species of Belostomatidae have been reported in Manitoba. Specimens of Benacus griseus (Say) have been taken in Winnipeg, Lethocerus americanus at Beausejour, Brandon, Falcon Lake, Millwood, Morden, Mulvihill, Red Rock Lake, Rennie, and Winnipeg. Specimens of Belostoma flumineum (Say) have been taken at Arnaud, Aweme, St. Elisabeth, and St. Norbert (Clark, 1926; Brooks and Kelton,

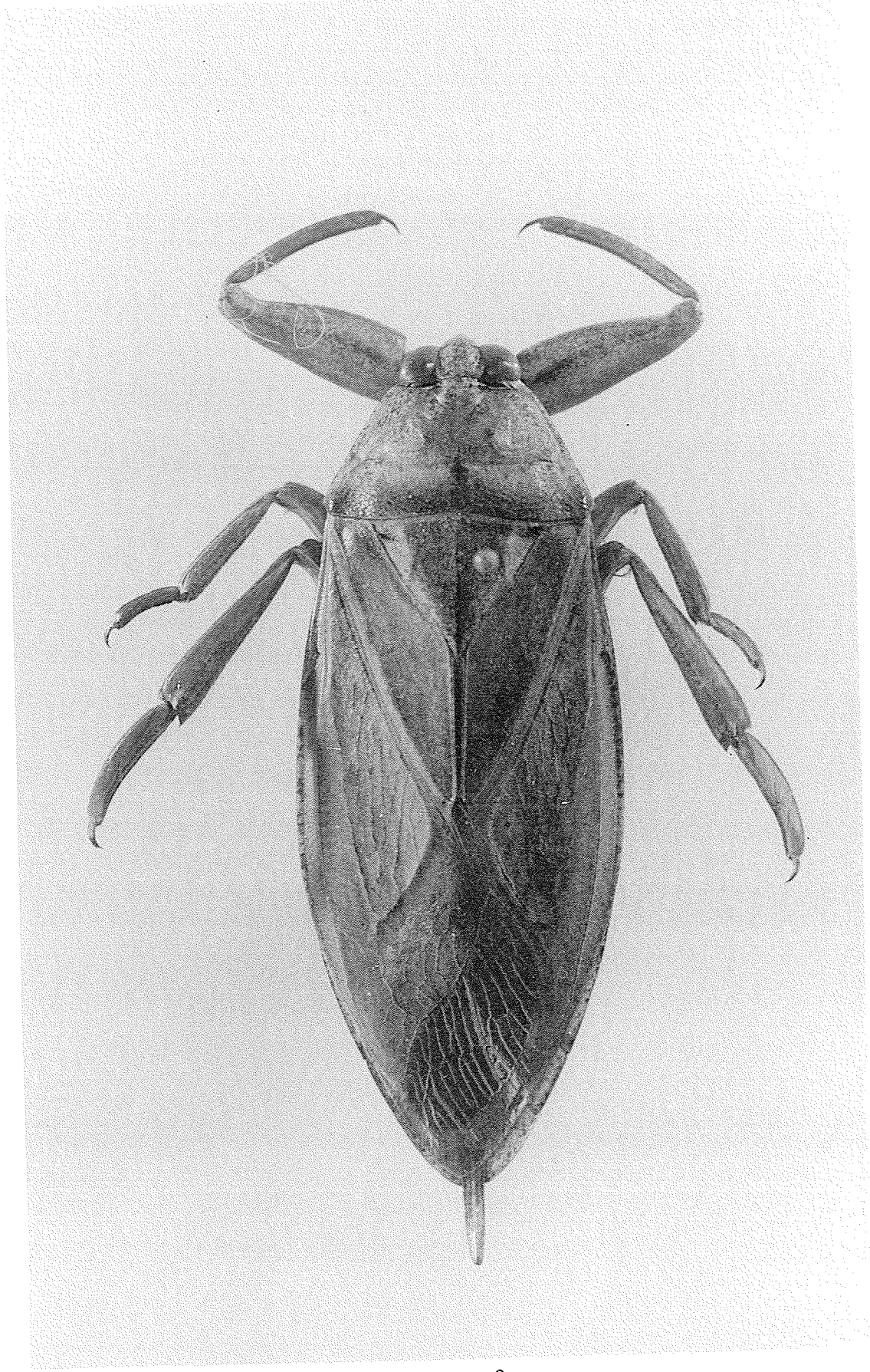

Figure 3
Adult female Lethocerus americanus (Leidy) - the giant water bug. The strap-like breathing appendages are to be seen protruding from under the hemielytra

1967). Specimens of Belostoma flumineum and Lethocerus americanus for this study were collected within the W.N.R.E. Controlled Area. At W.N.R.E., nymphs and adult L. americanus with their eggs, were abundant in the experimental ponds in 1967. No eggs, nymphs or adults were found in the ponds during 1968.

The procedure followed for rearing and feeding L. americanus in the laboratory has been described under Methods. Observations made on laboratory colonies will be described, because they afford some interesting comparisons with those of other workers.

EGG STAGE

Hoffman (1924) has quoted an observation made by Professor Parker who noted "giant water bugs guarding their eggs on a bank of small slough". No guardian adults were seen on any of the occasions when eggs were taken from the experimental ponds. Batches of eggs were obtained from clumps of algae, the stems of cattails (Typha sp.) growing in the margin of the radioactive pond, and from the buoys supporting the water temperature probes. Batches of eggs obtained from the non-radioactive pond were taken to the nearby field laboratory for hatching. Most batches of eggs began hatching 11 days after collection, and continued hatching until day 17. The average incubation time of eggs laid in the laboratory was 14 $\pm$ 3 days for L. americanus, and 11.5 $\pm$ 1 day for B. flumineum. A dramatic account of the egg hatching sequence has been given by Hungerford (1925), and personal observations are in agreement with his.

The emergence of young nymphs from eggs in some instances required as long as five hours. On emergence the nymphs were a pale white colour, and translucent. Within two hours, the dorsal surface became light green, then 2-3 hours later changed to light-brown when scelerotization was complete.

NYMPHS AND ADULTS

Development

First instar nymphs were quite active soon after hatching, and when disturbed swam off with the rapid, jerky, movement characteristic of the adult. At rest, the nymphs adopted the tail-up position, which is also characteristic of the adults. An air bubble developed shortly after hatching, which was secured under the posterior region of the hemielytra. The duration of the stadia of each instar are given in Table I. Rankin (1935) has described the various stages of L. americanus, and Torre-Bueno's description of the last molt of the insect is quite informative (Torre-Bueno, 1924). No reference to the duration of instars in B. flumineum were found in the literature.

It was shown by Rankin (1935) that in L. americanus the time spent in each instar increased when the insect was raised in the laboratory, compared to rearing in a natural environment. Table I shows that the duration of L. americanus instars raised in the field laboratory at Pinawa, was also longer than for L. americanus raised in a sedge-pool in Douglas Lake, Michigan, (Rankin, 1935). Rearing

TABLE I

Average length in days of egg incubation, and nymphal stages of L. americanus and B. flumineum

	Incubation	1st instar	2nd instar	3rd instar	4th instar	5th instar	Totals*
L. americanus							
Reared in laboratory at Pinawa	14.5	5.5	6.5	9.0	10.0	16.0	47.0
Reared in laboratory (1)	-	9.2	8.2	8.5	11.5	21.5	58.9
Reared in Sedge pool (1)	-	5.0	4.9	5.7	6.	11.8	33.4
B. flumineum							
Reared in laboratory at Pinawa	9.6	8.6	6.6	6.3	6.0	13.3	40.8
Reared in laboratory (2)	11.5	-	-	-	-	-	-

(1) from Rankin, 1935

(2) from Torre-Bueno, 1906

* Incubation time not included in totals.

conditions, particularly temperature, probably account for this difference. The laboratory development period of water bugs at Pinawa was shorter than that of those raised by Rankin in his laboratory (Table I). Again, in the absence of details it must be assumed that the respective rearing environments account for the observed differences. The duration of the period from first instar to adult molt for B. flumineum was 40.8 days, significantly less ($p = 0.05$) than that of L. americanus. Table I also shows the duration of each instar of the latter insect, reared in the laboratory, progressively increased from the first to the fifth instars. The stadial period of B. flumineum showed no significant change until the fourth instar.

Dimension of Nymphs

Table II gives the observed average length and maximum width of the various instars of L. americanus and B. flumineum, and compares these measurements with those reported by other workers (Torre-Bueno, 1906; Batchley, 1926; Cummings, 1933; Rankin, 1935; and Brooks and Kelton, 1967). The average length of female L. americanus taken at Pinawa was not significantly greater ($p = 0.05$) than that of males. It was not possible to determine from the measurements reported in Table II, if the length and width measurements of L. americanus and B. flumineum varied with locality of capture.

The length of femur, tibia etc., measurements of some nymphal and adult stage of L. americanus and B. flumineum reared at

TABLE II

Length and greatest width of various stages of L. americanus and B. flumineum - Pinawa species, and others. Dimensions of Pinawa species given as mean ± one standard error

Stage	Length - mm		Greatest Width - mm	
	Pinawa Species	Others	Pinawa Species	Others
L. americanus				
1st instar	10.0 ± 0.50	10(4)	5.5 ± 0.25	5(4)
2nd instar	14.5 ± 0.50	15(4)	7.3 ± 0.25	8(4)
3rd instar	19.8 ± 0.37	22(4)	9.8 ± 0.16	11(4)
4th instar	27.8 ± 0.62	31(4)	14.1 ± 0.35	14.5(4)
5th instar	38.3 ± 1.1	39(4)	18.1 ± 0.39	18(4)
Adult	54.7 ± 1.39	45-55(2,5) 42-62(3)	20.5 ± 0.48	16.5-24.(2,3)
B. flumineum				
1st instar	-	4-5(1)	-	2.4(1)
2nd instar	-	6-7(1)	-	3.4-3.7(1)
3rd instar	10.1 ± 0.25	8.5-9.(1)	5.5 ± 0.17	3.7-4.(1)
4th instar	14.0 ± 0.01	10-12(1)	7.8 ± 0.25	6.7(1)
5th instar	16.4 ± 0.37	15.7-17.(1)	8.5 ± 0.22	8.4-8.8(1)
Adult	22.1 ± 0.41	21-24		

(1) Torre-Bueno 1906

(2) Blatchley 1926

(3) Cummings 1933

(4) Rankin 1935

(5) Brooks and Kelton 1967

Pinawa, together with sample size and variation, are compiled in Appendices D and E. Examination of these appendices shows that several interesting correlations between changes in dimensions of a member through successive instars are possible. For example: Differences between the length of the femur of the fore-, mid-, and hind-legs, and the length of the tibia of the same legs in successive instars, were tested by Student's 't' test (Bailey, 1959). For L. americanus, the femur of the fore-, and hind-legs, and tibia of the fore-, and mid-leg are the same length in relation to each other, in each instar. Neither the femur or tibia of any leg relative to one another, was constant from instar to instar in B. flumineum.

Molting

L. americanus and B. flumineum molt five times before reaching the adult stage. The period of ecdysis is a critical one for most insects and aquatic Hemipterans are apparently no exception. Hungerford (1919), discussing the biology of the water boatman Palmocorisca buenoi Abbot remarked "under laboratory conditions, molting appears to be a precarious process". Hungerford (1924), studying a species of water scorpion Ranatra, came to the same conclusion. Losses as great as 20-25% in the laboratory colonies were sustained at each molt, especially during the molt to the fourth instar. The reason for this was not apparent. It was not possible to determine whether the death-rate at molting was higher for water

bugs fed non-radioactive tadpoles in the laboratory at Pinawa, than for those in Nature. Examination of the radioactive and non-radioactive ponds throughout the summer of 1967 yielded many specimens of L. americanus which apparently had died during molting. Measurements obtained from these instars corresponded with those of laboratory specimens which died during ecdysis. Hoffman (1924) made comparable observations, and he was unable to establish if the number of molting-deaths was higher among water bugs raised in captivity. In one instance, a nymph was seen molting to the 4th instar and trying to emerge backwards from the split skin. It died! Rankin's observations, however, indicate that the overall molting mortality is highest for laboratory-reared L. americanus. In the laboratory, 45% of his nymphs died in the 2nd instar and 18% in the 3rd instar. No deaths occurred before the 4th instar in nymphs reared in field-cages (Rankin, 1935).

General Observations

Frequently, nymph or adult water bugs ejected a dark-green colored liquid from the anal region, rather like the ink-cloud expelled by a squid when it has been disturbed or frightened. The liquid had a peculiar foul smelling "fishy" odor. Adult L. americanus have also been reported to make wheezing noises (Hungerford, 1925). In the laboratory at Pinawa, 3rd, 4th and 5th instar nymphs, and adults, were heard making distinct squeaking and scraping noises.

Hoffman (1924) has noted that adult L. americanus may overwinter. None of the marked adult water bugs returned to the radioactive pond in late September 1967, were found the following spring. However, prior to marking the adults for return to the pond at the end of summer, those in the laboratory appeared to become restless. Hitherto, the adults seldom made any attempt to escape from their rearing-trays when the cover was removed for cleaning or feeding. As autumn advanced, attempted escapes became more frequent.

Feeding Behavior

Earlier authors, for example Rankin (1935), have reported successfully feeding water bugs dead prey such as half-a-frog, or a piece of beefsteak. Attempts to induce water bugs reared in the laboratory to feed on recently killed tadpoles or fresh meat failed. Living tadpoles were the staple diet of L. americanus reared in the laboratory at Pinawa.

First instar nymphs, three days old and unfed since hatching, were offered live water boatmen as prey. The nymphs swam amongst the boatmen with apparent indifference. However, when nine live tadpoles, weighing between 10 and 20 mg were placed in a rearing-tray with 20 nymphs, the tadpoles were immediately attacked. As many as three and four nymphs grasped the same tadpole, quickly subduing it. The nymphs commonly covered a frantically swimming tadpole in the tray and attached themselves to the head, back, or

base of the tail. Those tadpoles attacked at the base of the tail were immobilized within two or three minutes regardless of the relative sizes of nymph and tadpole. It has been suggested that L. americanus secretes a poison which paralyzes the prey (Rankin, 1935). As was also reported by Hoffman (1924), a nymph often fed on a tadpole for as long as one hour, after which time nothing remained of the animal but a shriveled-up skin and some dark mud-like material in the digestive tract.

First and 2nd instar L. americanus frequently congregated together, clasping one another, a response which may have been caused by crowded conditions in the rearing-trays. On several occasions nymphs were seen to attack and feed on other nymphs. This behavior was frequently observed after nymphs had fed on tadpoles, and live prey was still accessible. Perhaps tadpoles did not provide an adequate diet. It was the cannibalistic behavior of L. americanus, which necessitated rearing the water bugs in individual trays.

Behavior Towards Prey

When a live tadpole was placed in a tray with a young nymph, it was immediately pursued. It appeared that the vigor of the pursuit was related to the nymph's hunger. Having fed on the first tadpole, the second and third tadpoles were not pursued as vigorously. Having chased, captured, and fed on one or two tadpoles,

the nymph adopted a waiting posture. It clung to the wood block floating in its tray with its forelegs out-spread, and only seized another tadpole if it swam within reaching distance.

A marked change was noted in the feeding behavior of older water bugs, especially those which had been placed in captivity as adults. Initially the adult hung downwards in the water, holding on to the wood-block with its hind-legs, raptorial fore-legs out-spread. When a tadpole swam past within striking distance, the insect quickly lunged forward, seized the prey, and began to feed. After 7-10 days in captivity, adults made no effort to lunge after prey. It seemed insects had become accustomed to having dinner served by forceps between the forelegs. Adults deprived of "table-service" for 3-4 days reverted to the former prey response.

On one occasion in the radioactive pond, a 4th instar L. americanus was observed grappling with a large adult Dytiscus beetle; the beetle was larger and twice as heavy as its assailant. The nymph remained at the surface of the water clutching the beetle with all its legs. Some 30 minutes later when the beetle had stopped struggling, the nymph slowly manoeuvred it into a position where the beak was inserted at the junction of the thorax and the elytra.

In captivity, it appeared that movement along was not sufficient to cause capture. Tactile perception, as well as movement, seemed to be important, as forcep-feeding of adults clearly

showed. A tadpole brushing against the fore-legs, but not the mid-, or hind-legs, of blinded adults evoked the characteristic lunging-clutching response.

In the late summer of 1967, a pond was found which contained large numbers of late instar and adult L. americanus and B. flumineum. The average depth of water in this pond was less than 30 cm. Adults were brought into the field laboratory and one of each species placed in large plastic trays along with a small clump of algae. No food was offered, yet after four days there was no evidence of aggressive behavior. B. flumineum were seen to swim between the fore-legs of L. americanus without invoking an attack. On being frightened, for example prodded with a glass rod, B. flumineum sought concealment beneath the larger L. americanus.

CHAPTER IV

UPTAKE AND ELIMINATION OF ^{137}Cs BY L. AMERICANUS

As described under Methods, with the exception of the preliminary experiments, the giant water bugs used in these experiments were raised in the laboratory. Eggs for rearing were taken from the experimental ponds. Nymph and adult L. americanus were maintained on a diet of tadpoles. To measure the uptake of radiocesium, the water bugs were fed tadpoles taken from the radioactive pond. The total-beta activity of these tadpoles averaged 1×10^4 cpm/g. Elimination of ^{137}Cs was studied by feeding non-labeled tadpoles to water bug nymphs or adults which had reached a steady-state body-burden of the nuclide. The average total-beta count rate of the non-labeled tadpoles was 8.2 ± 0.8441 (standard error) cpm/g.

In a preliminary experiment, adult L. americanus taken from natural ponds were fed radiocesium labeled tadpoles for ten days, then fed non-labeled tadpoles; by this time only two adults were alive. The daily increase, or decrease, of the radiocesium body-burden of individual insects was followed to establish techniques. The uptake and elimination measurements are given in Figure 4, where the fraction of the steady-state level of ^{137}Cs has been plotted against time in days. The biological half-life (T_b) of uptake and elimination was estimated to be about eight days (Fig. 4).

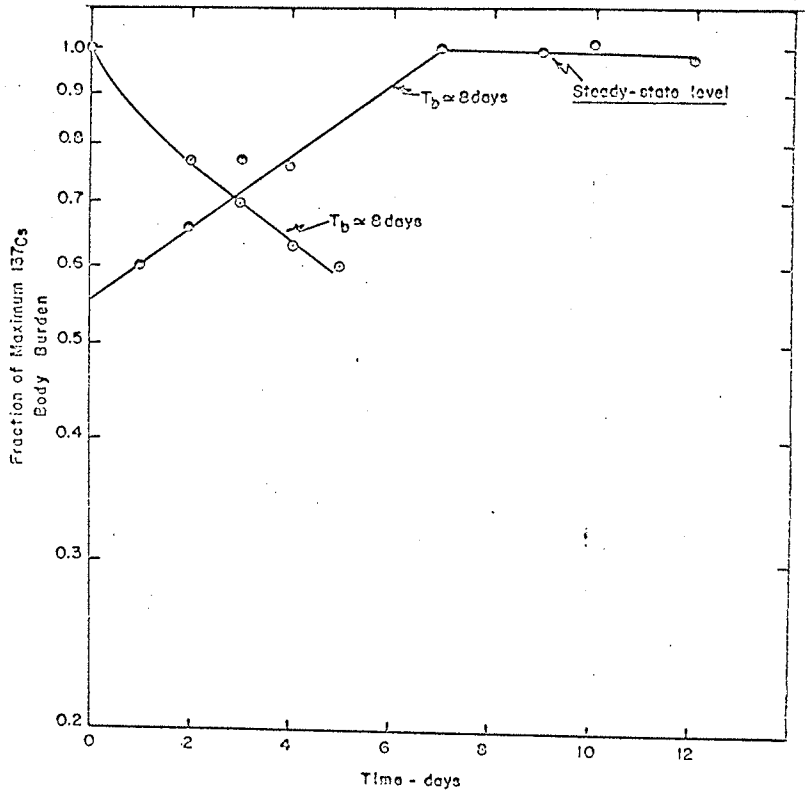

Figure 4

Uptake and elimination of ^{137}Cs by adult L. americanus captured in an outdoor pond, and fed radioactive tadpoles in the laboratory. ● - uptake, ○ - elimination, T_b = biological half-life.

This preliminary experiment was a prelude to the uptake experiments which followed, and it provided solutions to problems associated with feeding and counting water bugs.

RESULTS AND DISCUSSION

No. 1 Uptake Experiment

The preliminary experiment was repeated using a group of ten second instar L. americanus nymphs obtained from eggs which hatched in the laboratory during 5-7 July 1967. The temperature of the water in which the water bugs were reared fluctuated between 17°C and 20°C for the duration of the measurements. This fluctuation was comparable to that of the out-door pond from which the eggs were obtained. For convenient reference, this experiment henceforth will be referred to as "No. 1 Uptake". It has been noted previously that the attrition rate experienced raising water bugs, is high. No. 1 Uptake experiment was stopped when there were only four adults remaining from the original group of ten.

The results of No. 1 Uptake experiment are recorded in Table III, where mean weights and radiocesium activities are reported with one standard deviation (S.D.) of the measurement. Radiocesium activities are reported in counts per minute (cpm). All attempts to measure the uptake of ^{137}Cs by first instar nymphs were unsuccessful, because the counting statistics were unsatisfactory. Time and counting rate data presented in Table III are reproduced

TABLE III

Uptake of ^{137}Cs by various instars of L. americanus - No. 1 Uptake experiment. Ten first instar nymphs obtained from eggs hatched in the laboratory 5-7 July, 1967. Nymphs allowed to moult to second instar before radiocesium-labeled tadpoles were fed. ^{137}Cs activity reported in counts per minute (CPM) ± one standard deviation. Only four insects survived to adult stage

1 Time-days	2 Instar	3 Mean wt. g	4 Mean activity CPM	5 Mean CPM/g 4/3	6 Remarks
1	2nd	0.088 ± 0.011	74 ± 37.23	836	
2	2nd	0.088 ± 0.011	63 ± 24.88	719	
5	2nd	0.141 ± 0.078	183 ± 8.34	1298	
5	3rd	0.215 ± 0.025	93 ± 20.07	432	
7	3rd	0.217 ± 0.027	86 ± 27.28	396	2 died
9	3rd	0.246 ± 0.035	246 ± 34.95	465	
13	3rd	0.331 ± 0.037	213 ± 49.10	643	2 died
13	4th	0.569 ± 0.040	222 ± 30.05	390	
16	4th	0.752 ± 0.039	703 ±105.10	935	
18	4th	0.909 ± 0.043	604 ± 35.35	664	1 died
19	4th	0.955 ± 0.045	713 ± 61.89	747	
20	4th	0.938 ± 0.050	510 ± 25.23	545	
22	4th	0.920 ± 0.045	400 ± 38.24	435	
25	5th	1.32 ± 0.090	307 ± 60.73	233	
28	5th	1.54 ± 0.085	440 ± 82.10	286	
31	5th	1.97 ± 0.095	1300 ±145.48	660	1 died
35	5th	1.95 ± 0.095	1410 ±300.25	720	
38	5th	2.18 ± 0.105	840 ± 60.84	385	
41	5th	2.20 ± 0.085	660 ± 82.66	300	
43	Adult	3.17 ± 0.150	481 ±104.88	152	
50	Adult	2.89 ± 0.120	607 ±122.91	210	
57	Adult	2.75 ± 0.150	400 ±117.73	145	
64	Adult	2.96 ± 0.175	517 ±103.62	175	
70	Adult	3.10 ± 0.208	800 ±160.53	258	
78	Adult	3.29 ± 0.207	632 ±204.62	192	Adults returned to radioactive pond
6	(2nd	0.047 ± 0.004	5 ± 1.64	102	
7	(exuvia	0.047 ± 0.004	16 ± 2.83	340	

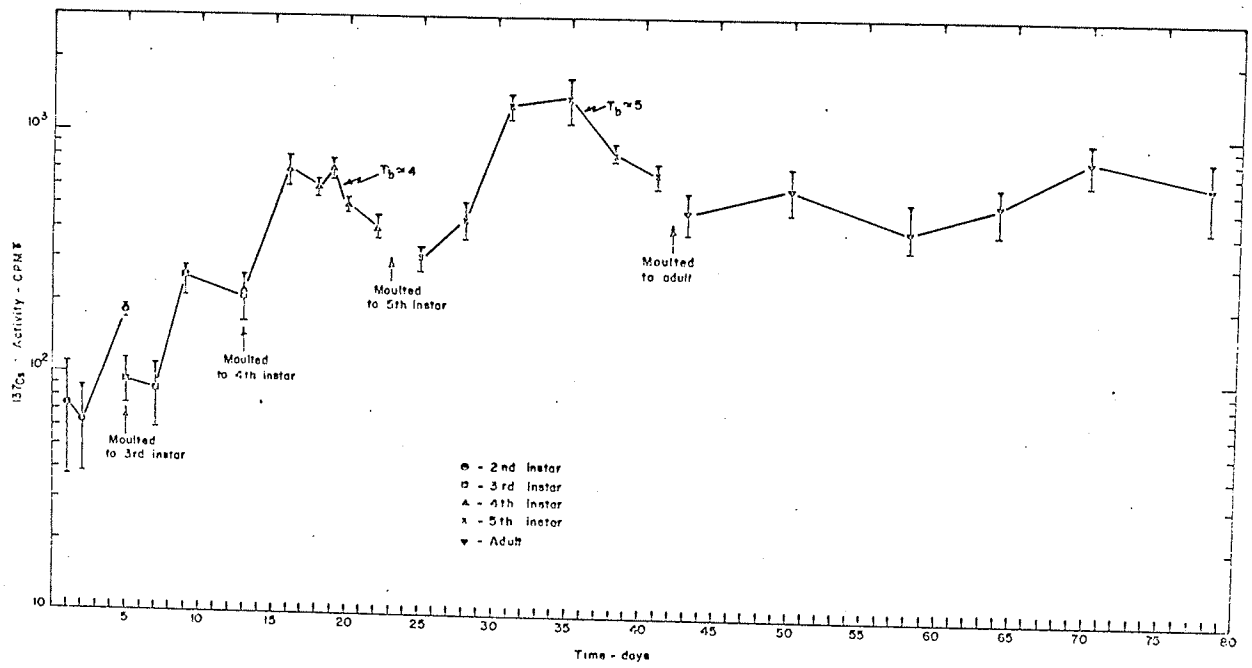

Figure 5

No. 1 Uptake by L. americanus. Uptake of ^{137}Cs by various instars fed ^{137}Cs labelled tadpoles. Means ± one standard deviation are plotted.

graphically in Figure 5, where it will be noted that a drop in radiocesium activity, particularly pronounced in the fourth and fifth instars, occurred prior to each molt. It is significant that the biological half-life of this drop or decrease approximates that observed in subsequent experiments designed to measure the elimination rate of radiocesium. The graphs in Figure 5 suggest that the rate of uptake of ^{137}Cs by second, third, fourth and fifth instar water bugs is between two and two and one half days. Unfortunately, all attempts to establish this value by more frequent counting were unsuccessful; the additional handling increased mortality.

No. 2 Uptake Experiment

To obtain a better estimate of the elimination rate of ^{137}Cs by L. americanus, a second group of nymphs obtained from eggs hatched in the laboratory on 11/12 July 1967, were fed radiocesium labeled tadpoles. After reaching steady-state in the third instar, the nymphs were fed non-labeled tadpoles. As the nymphs molted shortly after this transfer, it was not possible to measure the third instar elimination-rate. However the measurements made during this experiment, recorded in Table IV, do allow a satisfactory determination of the fourth instar elimination rate to be made. The measurements reported in Table IV are shown graphically in Figure 6, where it will be seen that the estimated biological half-life of

TABLE IV

Uptake and elimination of ^{137}Cs by various instars of L. americanus - No. 2 Uptake experiment. Ten first instar nymphs obtained from eggs hatched in the laboratory 11-12 July, 1967. Radioactive tadpoles fed to first instar. ^{137}Cs activity reported in counts per minute (CPM) ± one standard deviation

1 Time-days	2 Instar	3 Mean wt. g	4 Mean activity CPM	5 Mean CPM/g 4/3	6 Remarks
0	1st	0.178 ± 0.057	36 ± 0.700	202	measured prior to feeding active tadpoles
1	2nd	0.154 ± 0.008	139 ± 5.00	905	2 died
1	3rd	0.258 ± 0.019	180 ± 26.16	698	1 died
4	3rd	0.283 ± 0.018	158 ± 29.80	558	
6	3rd	0.323 ± 0.028	221 ± 49.04	684	2 died
7	3rd	-	-		began feeding non-labeled tadpoles
8	4th	0.496 ± 0.131	165 ± 48.14	333	
9	4th	0.540 ± 0.044	153 ± 42.42	283	
10	4th	0.594 ± 0.052	116 ± 28.28	195	
11	4th	0.593 ± 0.065	108 ± 42.42	182	
12	4th	0.641 ± 0.091	105 ± 4.95	164	
14	4th	0.682 ± 0.034	65 ± 10.60	95	
16	4th	0.694 ± 0.040	43 ± 22.62	62	
19	4th	0.900 ± 0.098	29 ± 5.65	32	
22	4th	1.03 ± 0.140	23 ± 4.48	22	
26	5th	1.49 ± 0.080	11 ± 0.10	7	
29	5th	1.97 ± 0.08	23 ± 1.50	12	
34	5th	2.43 ± 0.08	22 ± 1.50	9	
41	Adult	2.74 ± 0.09	18 ± 4.10	7	
48	Adult	2.79 ± 0.09	25 ± 5.00	9	

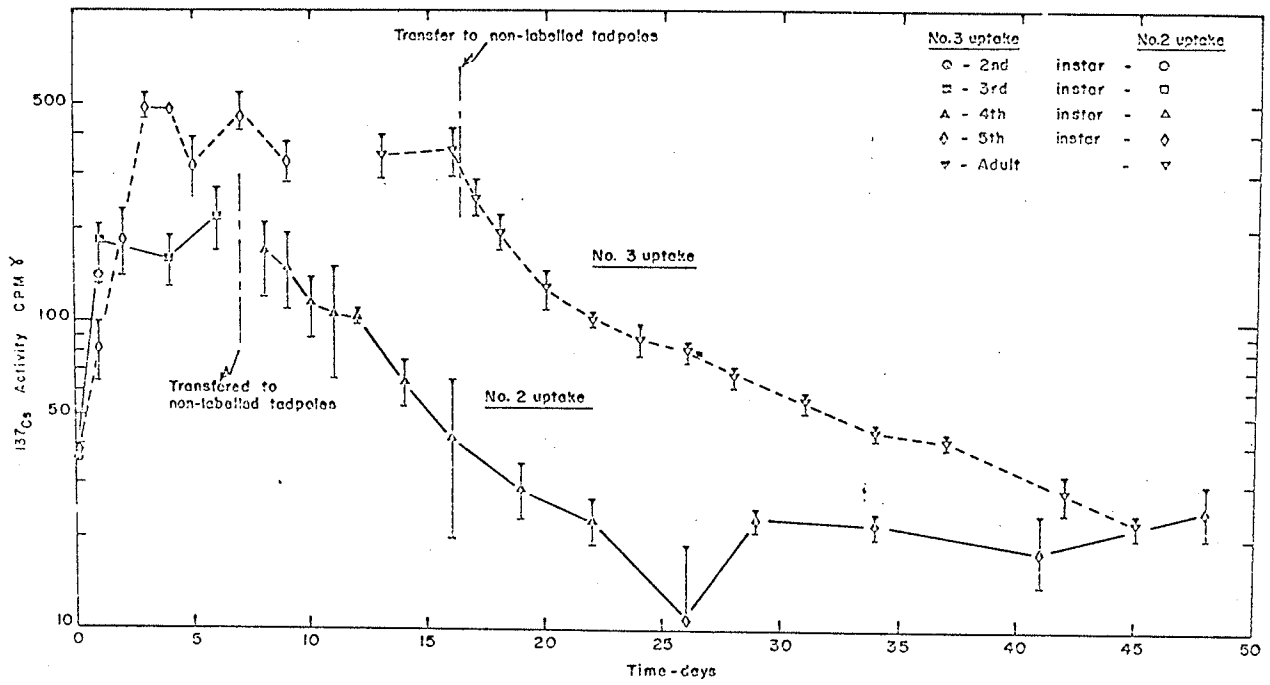

Figure 6

Nos. 2 and 3 Uptake by L. americanus. Uptake and elimination of ^{137}Cs by 4th instar nymphs, and adults. Nymphs were fed ^{137}Cs labelled tadpoles until steady-state level was reached, then fed unlabelled tadpoles. Plotted points are means ± one standard deviation.

^{137}Cs by the second instar was again about two days. The decrease in radiocesium body-burden of the fourth instar nymphs is plotted as a fraction of the initial ^{137}Cs steady-state level against time, in Figure 7. The regression coefficient of this curve is -0.9908. From this curve the biological half-life was calculated to be 4.5 days. The linearity of the curve in Figure 7 indicates that radiocesium was being eliminated from one major body compartment only, or conversely, all compartments eliminated the nuclide at the same rate.

No. 3 Uptake Experiment

A group of fifth instar nymphs, also obtained from L. americanus eggs hatched in the laboratory 11-12 July 1967, were fed ^{137}Cs labeled tadpoles until reaching the steady-state level. At this time they were fed non-labeled tadpoles. Mean wet weights of nymphs and radiocesium levels are reported in Table V, and are also plotted using the open symbols, in Figure 6. It will be seen from this figure that the uptake-rate of ^{137}Cs, expressed in terms of the biological half-life, was about two days. Again, the nymphs molted shortly after being transferred to non-labeled tadpoles. These measurements are plotted as fraction of the steady-state activity in Figure 8. This figure shows the elimination rate of ^{137}Cs by adult L. americanus. In contrast to that of fourth instar nymphs shown in Figure 7, it will be seen in Figure 8 that the elimination rate of the adults was not constant throughout the 30

day period by observation. The linear portion of the curve, marked 'A' in Figure 8, has a regression coefficient equal to -0.9975. This part of the curve was extrapolated to the ordinate axis (see broken line in Figure 8), and the appropriate increments subtracted from the activities measured during the interval from the sixteenth to the twenty-second day. The remainders are plotted as curve 'B' in Figure 8, from which a T_b = 1.2 days was measured. Curve 'A' reflects a T_b = 10.8 days. The data plotted in Figure 8 suggest that, in contrast to fourth instar nymphs, radiocesium is eliminated at two distinct rates by adult L. americanus.

The body-burden of radiocesium in L. americanus decreased before molting which indicates that juvenile water bugs stopped eating prior to ecdysis. It has been suggested by Wigglesworth (1965) that the immature stages of insects stop eating prior to molting. Examination of Figure 5 will show that the decrease began, in the case of 4th instar nymphs, as much as four days before molting. During the period when the ^{137}Cs body-burden was decreasing, the weight of individual nymphs did not change significantly (Table III). Specific activity, that is radiocesium activity in cpm/weight (Table III, column 5) also decreased. If specific activity is considered to be a reflection of radionuclide concentration per unit mass, then this observation indicates that the 4th and 5th instar nymphs had begun to reorganize tissue in preparation for ecdysis about four days prior to molting. If this hypothesis is

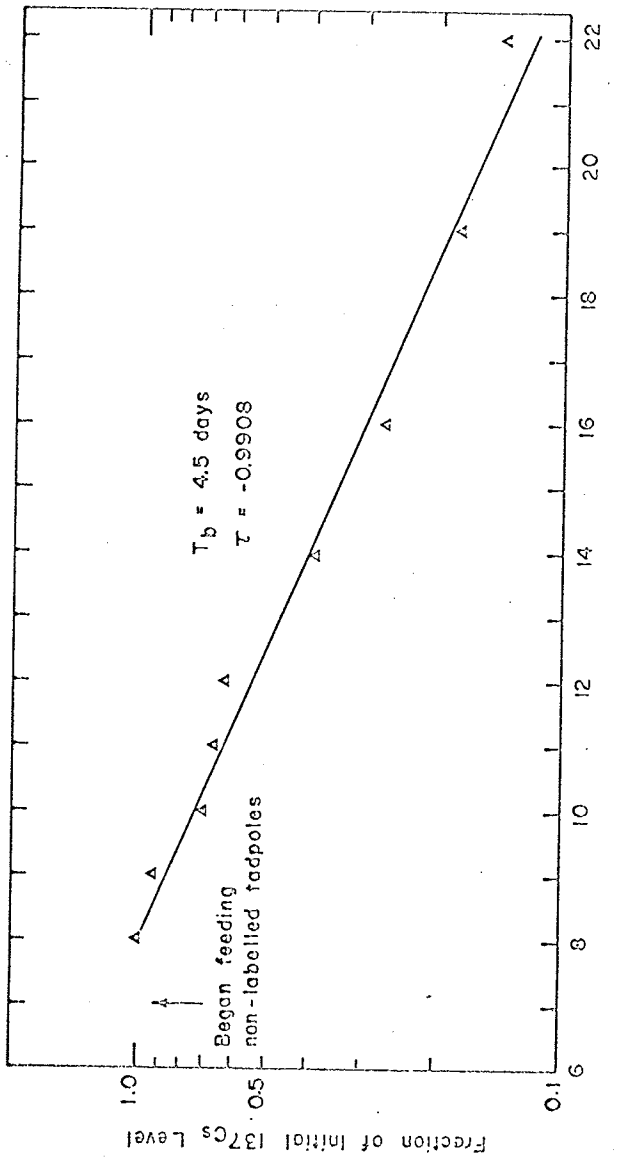

Figure 7

Elimination of ^{137}Cs by 4th instar L. americanus. Mean ^{137}Cs activities from Table IV are shown as logarithm of fraction of steady-state level. Nymphs were fed unlabelled tadpoles. T_b = biological half-life, r = correlation coefficient.

TABLE V

Uptake and elimination of ^{137}Cs by L. americanus - No. 3 Uptake experiment. Ten 5th instar nymphs obtained from eggs hatched in the laboratory 11-12 July, 1967. Nymphs fed non-radiocesium-labeled tadpoles after completing adult moult. ^{137}Cs activity reported in counts per minute (CPM) ± one standard deviation

1 Time - days	2 Instar	3 Mean wt. g	4 Mean activity CPM	5 Mean CPM/g 4/3	6 Remarks
0	5th	1.45 ± 0.387	38 ± 2.13	26	measured prior to feeding active tadpoles
1	5th	1.47 ± 0.402	83 ± 18.78	56	
2	5th	1.58 ± 0.413	185 ± 45.80	117	
3	5th	1.86 ± 0.289	494 ± 47.13	266	
4*	5th	1.68 -	478 -	285	*Died during night
5	5th	1.95 ± 0.324	321 ± 71.81	165	
7	5th	2.01 ± 0.335	477 ± 68.47	237	
9	5th	2.06 ± 0.514	330 ± 50.37	160	1 dead
13	Adult	2.60 ± 0.321	342 ± 53.34	132	1 dead
16	Adult	2.43 ± 0.357	355 ± 60.68	146	began feeding non-labeled tadpoles
17	Adult	2.73 ± 0.499	254 ± 32.46	93	
18	Adult	2.51 ± 0.557	195 ± 24.63	78	
20	Adult	2.36 ± 0.421	130 ± 16.41	55	
22	Adult	2.38 ± 0.408	102 ± 3.39	43	
24	Adult	2.47 ± 0.342	88 ± 9.54	36	
26	Adult	2.46 ± 0.412	80 ± 6.10	33	
28	Adult	2.47 ± 0.343	67 ± 4.52	27	
31	Adult	2.34 ± 0.334	55 ± 3.91	24	
34	Adult	2.40 ± 0.310	44 ± 1.50	18	
37	Adult	2.47 ± 0.432	41 ± 2.08	17	
42	Adult	2.41 ± 0.227	28 ± 3.86	12	
45	Adult	2.35 ± 0.037	22 ± 2.16	9	All died overnight
18	5th exuvia	0.037 ± 0.005	46 ± 24.34	1243	

*Died overnight - single measurement

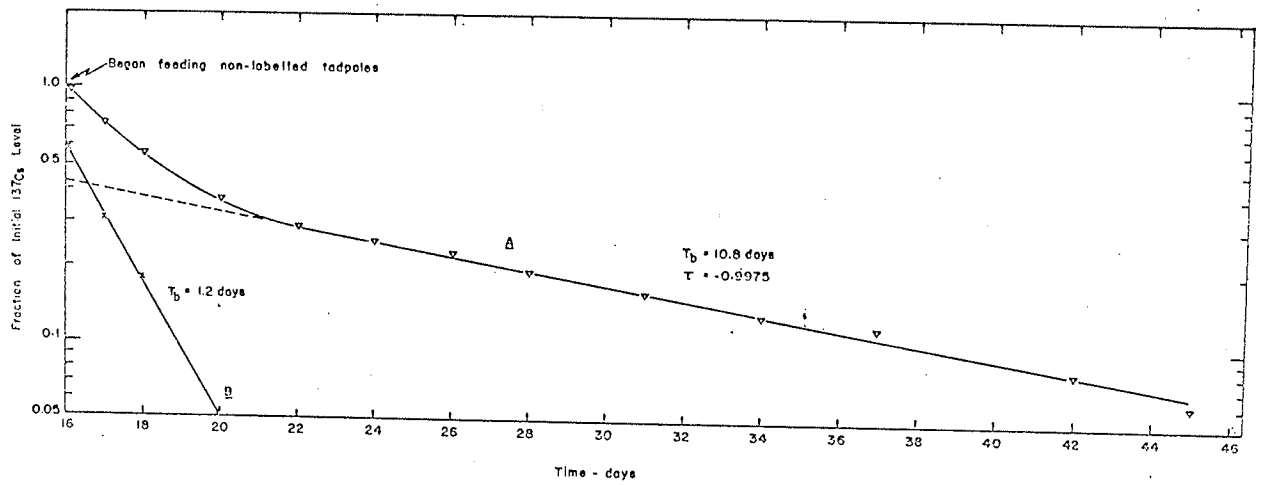

Figure 8

Elimination of ^{137}Cs by adult L. americanus. Mean ^{137}Cs activities from Table V are shown as logarithm of fraction of steady-state level. Adults were fed unlabelled tadpoles. T_b = biological half-life, r = correlation coefficient.

correct, the radio-label technique offers a simple method for determining the onset of this important physiological activity.

The elimination of radiocesium by 4th instar and adult L. americanus is shown in Figures 7 and 8, where the logarithm of the mean fraction of steady-state ^{137}Cs activity $\pm$ one standard deviation (N = 10) is plotted against time in days. It will be seen from Figure 7 that radiocesium was eliminated by the 4th instar nymphs at a single rate, T_b = 4.5 days. The adult water bugs eliminated their ^{137}Cs body-burden at two rates, T_b = 10.8 days (Curve 'A', Figure 8), and T_b = 1.2 days (Curve 'B', Figure 8). The latter value is suggestive of the insect's 24 hour feeding cycle and is assumed to represent the biological half-life of non-assimilated radiocesium. A single elimination rate is indicative of complete assimilation of ^{137}Cs label from the gut (Crossley and Howden, 1961; Crossley, 1963), therefore, 4th instar L. americanus are assumed to have an assimilation fraction equal to unity. The same conclusion can not be made for the adult insect.

For the adult water bug, consider a two compartment model in which the total amount of labeled food ingested (I) comprises the amount entering the body compartment (or haemocoele) and that which is not assimilated. The insect's gut is a probable location of the unassimilated component of I. The body compartment shall be designated with the sub-script 'b', and the unassimilated or gut compartment with the sub-script 'g'.

Now the rate of entry of ^{137}Cs label into the body compartment is:

$\frac{dI.F}{dt}$, where F is the fraction of I that enters the body compartment.

$$\therefore \frac{dI}{dt} = \frac{dI.F}{dt} + \frac{dI(1 - F)}{dt}$$

That is, the total ingestion rate equals the sum of the entry rates into compartments 'b' and 'g'.

Let the amounts of ^{137}Cs in each compartment be Q_b and Q_g, and the exit rates from compartments 'b' and 'g' be exponential with elimination constants '$-\lambda_b$' and '$-\lambda_g$'

Thus:
$$\frac{dQ_b}{dt} = \frac{dI.F}{dt} - \lambda_b.Q_b, \text{ and} \qquad \ldots\ldots\ldots \text{ I}$$

$$\frac{dQ_g}{dt} = \frac{dI(1 - F)}{dt} - \lambda_g.Q_g$$

At steady-state:

$$\frac{dQ_b}{dt} = 0, \text{ and } \frac{dQ_g}{dt} = 0$$

$$\therefore \frac{dI.F}{dt} = \lambda_b.Q_b, \text{ and } \frac{dI(1 - F)}{dt} = \lambda_g.Q_g$$

When feeding of labeled tadpoles ceased, I = 0, and $\frac{dI}{dt} = 0$

$$\therefore \frac{dQ_b}{dt} = -\lambda_b.Q_b, \text{ and } \frac{dQ_g}{dt} = -\lambda_g.Q_g$$

Similarly, from Equation I:

$$\frac{F.dI}{dt} = -\lambda_b.Q_b, \text{ and } \frac{(1 - F).dI}{dt} = -\lambda_g.Q_g$$

$$\therefore F = \frac{\lambda_b.Q_b}{(\lambda_b.Q_b + \lambda_g.Q_g)} \quad \ldots\ldots\ldots\ldots\ldots \text{ II}$$

Values for λ_b and λ_g can be calculated from the slopes of curves 'A' and 'B', Figure 8. They are $\lambda_b = 0.065$, and $\lambda_b = 0.613$. Extrapolation of these curves (Figure 8) to 16 days, i.e., $I = 0$, gives values for Q_b and Q_g in terms of fraction of Q, the specific activity of the whole insect at steady-state. From Figure 8, $Q_b = 0.42Q$, and $Q_g = 0.58Q$.

Substituting these values in Equation II:

$$F = \frac{(0.065 \times 0.42)Q}{(0.065 \times 0.42 + 0.613 \times 0.58)Q} = \underline{\underline{0.07}}$$

If the assumption that curve 'A' in Figure 8 represents the elimination rate of ^{137}Cs from the body compartment is correct, adult L. americanus in the laboratory assimilated less than one-tenth of the radiocesium label and probably less than 10% of the food which they ingested. The adult assimilation fraction is in marked contrast with that of 4th instar nymphs.

Having estimated values for 'F', the fraction of ingested food assimilated into the body compartment, the weight of tadpoles consumed daily to maintain the steady-state level of ^{137}Cs may be calculated from the model:

Recalling that $I = \frac{Q.\lambda_b}{F}$ III

where, I = ingestion or feeding rate,

F = fraction of ingested prey assimilated into the body compartment,

λ_b = elimination rate constant of body compartment, and

Q = total body-burden, i.e., specific activity of 4th instar or adult water bug.

Considering first the 4th instar L. americanus, pertinent data are:

Q = 780 cpm/g nymph (Table III)

T_b = 4.5 days (Figure 7)

F = 1

$\lambda_b = \frac{\ln 2}{T_b} = \frac{0.693}{4.5} = 0.15$

Substituting in Equation III:

$I = \frac{780 \times 0.15}{1} = 117$ cpm/g nymph/day

That is, each 4th instar water bug in order to maintain its steady-state level in the laboratory assimilated 117 cpm/g nymph/day of ^{137}Cs. The specific activity of the tadpoles which were fed to the nymphs and adults was 1×10^4 cpm/g. Therefore the weight of tadpole consumed to supply this amount of ^{137}Cs activity was:

$\frac{117}{1 \times 10^4} = \underline{0.012 \text{ g tadpole/g nymph/day}}$

For L. americanus, the pertinent data are:

Q = 200 cpm/g adult (Table III)

T_b = 10.8 days (Curve 'A', Figure 8)

F = 0.07

$\lambda_b = \frac{\ln 2}{T_b} = \frac{0.693}{10.8} = 0.064$

Substituting in Equation III:

$$I = \frac{200 \times 0.064}{0.07} = 182 \text{ cpm/g adult/day}$$

Tadpole consumption required to maintain this rate of ^{137}Cs intake was:

$$\frac{182}{1 \times 10^4} = \underline{0.018 \text{ g tadpole/g adult/day}}$$

The tadpole consumption rates required to maintain the steady-state ^{137}Cs body-burden of the nymphs and adults, are in good agreement. If the average consumption rate of nymphs and adults is assumed to be 0.015 g tadpole/g insect/day, and the mean weight of the tadpoles to be 0.16 g, then the fraction of prey ingested was about one-tenth.

The difference between the fraction of food ingested by terrestrial insects and isopods, and that of adult water bugs is remarkable. Reichle (1967) has reported radiocesium assimilation factors of 0.7 and 0.87 for four species of terrestrial isopods, and these are one order of magnitude higher than the value obtained for adult L. americanus. The basic assumptions of the model which has been used are: 1) The total ingestion rate is equal to the sum of the body compartment ingestion rate and the so-called gut compartment, and 2) radiocesium is not preferentially concentrated in either compartment. As was pointed out by Crossley (1963), ingestion rate is a function of the fraction of the ingested food

that is assimilated. It is apparent from the foregoing calculations that another parameter should be introduced into the model to compensate for the fact that L. americanus, unlike adult beetles, do not consume their prey completely. This factor which may be called the fraction of food ingested, would permit an estimate to be made of the number of prey killed.

Reichle (1967) has demonstrated radiocesium turnover in isopods to be a function of metabolic rate. The assimilation rates measured for 4th instar and adult L. americanus, 1.0 and 0.07, show that the metabolic rates of nymphs and adults are quite different. The specific activity measurements (Table III) support this conclusion.

It will be recalled that the biological half-life of ^{137}Cs was smaller for 4th instar water bugs, than for the adults. Crossley (1966) also found the biological half-life of radiocesium was less for the larvae of Chrysomela knabi (Brown) than in the adult stage of this terrestrial beetle. In addition, Crossley (1966) found that the biological half-life was affected by the age of the adult. Newly emerged adult beetles did not eliminate ^{137}Cs as quickly as did overwintered adults. The age of adult L. americanus had no demonstrable effect on the rate of radiocesium elimination by that species. Crossley (1963) has also suggested that the biological half-life of radiocesium in an insect increases with the size of the insect. L. americanus adults are one of the largest

species of insects found in North America, and its 10.8 day biological half-life supports Crossley's conclusion. The giant water bug studies which have been described clearly show, when the ^{137}Cs elimination rate measurements are compared with those reported for other insects (Crossley and Pryor, 1960; Crossley and Schnell, 1961; and Crossley, 1966), that large differences between the biological half-lives of radiocesium in aquatic and terrestrial insects are to be expected. Aspects of the L. americanus studies have been reported in the literature (Guthrie and Brust, 1969).

CHAPTER V

UPTAKE OF ^{137}Cs BY *AEDES AEGYPTI* AND *A. ATROPALPUS* (DIPTERA: CULICIDAE)

INTRODUCTION

Studies of insect metabolism using radionuclides are being actively pursued at the Oak Ridge National Laboratories, to which the annual progress reports of the laboratory bear witness. Workers at this and other national laboratories in the United States of America, are engaged in a variety of ecological investigations employing radionuclide tracers and techniques. Russian workers, notably Peredel'skii and Bogatyrev (1959a and b), and Getsova and Volkova (1962), are actively engaged in studying radio-contamination of insects and their environment by fission products. Pendleton (1957, 1962) has made reference to the uptake of ^{137}Cs by insects living in aquatic habitats, and Crossley and Schnell (1961) have compared the elimination rates of ^{137}Cs and ^{90}Sr by grasshoppers. Phosphorous-32 has been used to study feeding behavior of adult *Aedes aegypti* (Schmidt and Smith, 1962), and the uptake of this nuclide by the same species has been investigated by Quraishi (1968). Several nuclides, ^{144}Ce, ^{89}Sr, and ^{32}P for example, have been used to 'tag' mosquitoes for the purpose of studying their dispersal patterns (O'Brien and Wolfe, 1963). It appears from the literature, however, that the accumulation of ^{137}Cs by mosquitoes has not engaged the attention of investigators.

Two mosquito species, Aedes aegypti and A. atropalpus, were used to study the uptake of radiocesium by an aquatic Dipteran. Their biology has been described by Horsfall (1955). The feeding, rearing, and counting techniques employed have been described under Methods. Nevertheless some amplification of the mosquito methods is in order. The amount of radiocesium in individual larvae, pupae and adults, although reported as counts per minute (cpm) ^{137}Cs, was measured as total-beta activity in terms of ^{40}K equivalent. The rationale for measuring total-beta activity in this manner, together with the necessary counter calibration techniques, have been described (Guthrie and Grummitt, 1963). The background or natural radioactivity content of larvae reared on non-labeled food was found to be 0.1 cpm. It was justifiable, therefore, to attribute any beta activity exceeding this amount to ^{137}Cs. A comparison of total-beta activities measured in larvae reared on radiocesium-labeled food, with the accumulated amounts of ^{137}Cs determined by radiochemical analysis (Guthrie and Grummitt, 1963), demonstrated their one to one correspondence; coefficient of correlation (r) = 0.9984.

Radiocesium labeled dog food for feeding A. aegypti larvae, was prepared by stirring coarsely ground Gaines dog-meal in distilled water containing 10^4 cpm/ml carrier free ^{137}Cs, for 15 minutes. After stirring, the meal was allowed to settle and the supernatant drawn off. The meal was then washed three times with distilled

water, to remove radionuclide adhering loosely to the surface. The food was then spread out on a large enamel tray to dry at room temperature. When dry, the food was re-ground, and stored in screw-top glass jars in a refrigerator. The specific activity of the labeled A. aegypti food was measured radiochemically and found to be about 10^6 cpm/g dried food. This batch of food was used in all the radiocesium uptake by A. aegypti experiments. As previously described, a fish rearing food was used to feed A. atropalpus larvae. This food was labeled with carrier free ^{137}Cs, in a manner similar to that described for the A. aegypti labeled-food, with the exception that it received only one washing with distilled water after contacting with the radiocesium solution. Washing three times with distilled water removed too much nutrient, and the larvae did not thrive as well as those reared on unwashed food. The fish-rearing food sorbed more radiocesium than the dog-meal, the specific activity of the former being at least 10^7 cpm/g dry weight.

RESULTS AND DISCUSSION

No. 1 Uptake of Radiocesium by Aedes aegypti

A preliminary uptake experiment, designated No. 1 Uptake by A. aegypti, was run to establish methodology. This experiment was successful and served as the pattern for all subsequent uptake experiments using mosquitoes. Plastic rearing trays were set up, each containing 100 first instars randomly selected from newly hatched larvae. These larvae were reared at 20°C, and the tray-water changed

and the larvae fed labeled dog-meal, every 24 hours. Random samples of six larvae were selected for individual total-beta activity measurement, every hour for the first six hours, at 24 hours, 30 hours, and daily thereafter. The mean activities ± one standard deviation of these samples are given in Table VI.

It will be seen from Table VI that there was a steady increase in activity per larva, as larvae passed through successive sampling periods. A steady-state body-burden of radionuclide was reached after 168 hours, when third instar larvae were present. It will also be seen from Table VI that the radiocesium content per adult was less than that per larva. The concentration of radioactivity in the pupal exuvia was quite small. The latter finding is in agreement with that of other workers who have studied the uptake of ^{32}P (Riordan, 1965; Quraishi, Brust and Lefkovitch, 1966).

The data given in Table VI are reproduced graphically in Figure 9. In this figure, the logarithm of ^{137}Cs activity is plotted against sampling time in hours. In contrast to the uptake measurements made on L. americanus, no detectable decrease in the body-burden of the larvae occurred prior to molting. The uptake-rate of larvae sampled up to 140 hrs was uniform, the same linear equation describing all points within this period. The broken-line marked 'A' in Figure 9 is the least-squares fit of this equation. Its pertinent statistics are:

TABLE VI

No. 1 uptake of ^{137}Cs by Aedes aegypti. Larvae fed ground dog-chow labelled with ^{137}Cs. Specific activity of food = 10^6 CPM/g. Rearing temperature = 20°C. ^{137}Cs given in mean counts per minute (CPM) ± one standard deviation total - Beta activity

Sampling time - hrs	Mean Activity CPM	Remarks
1	0.90 ± 0.34	N = 5
2	0.59 ± 0.31	N = 6
3	0.17 ± 0.16	N = 5
4	0.18 ± 0.16	N = 6
5	0.35 ± 0.24	N = 6
6	0.40 ± 0.28	N = 6
24	0.96 ± 0.65	N = 6
30	2.0 ± 1.11	N = 6
48	4.9 ± 1.20	N = 6
71	12.9 ± 4.75	N = 6
98	23 ± 3.53	N = 6
98	49 ± 9.82	N = 6
120	168 ± 57.5	N = 6
144	348 ± 103	N = 6
168	546 ± 124	N = 6
192	519 ± 96.6	N = 6
218	437 ± 89.3	N = 6
246	540 ± 171	N = 6
268	474 ± 79.9	N = 6
293	323 ± 67.4	N = 6
312	340 ± 72.5	N = 5
336	305 ± 72.1	N = 6
336	11.6 ± 1.72	N = 6
268 ♂ pupae	160 ± 28.5	N = 6

TABLE VI Cont'd.

Sampling time - hrs		Mean Activity CPM		Remarks
317	♂ pupae	140	± 25.9	N = 5
363	♂ pupae exuvia	8.0	± 5.93	N = 12
268	♀ pupae	348	± 63.6	N = 6
317	♀ pupae	270	± 51.1	N = 6
363	♂ adults	142	± 7.76	N = 4
435	♂ adults	66	± 15.6	N = 5
460	♂ adults	34	± 16.5	N = 6
363	♀ adults	295	± 63.1	N = 2
435	♀ adults	148	± 28.3	N = 6
460	♀ adults	146	± 25.6	N = 5

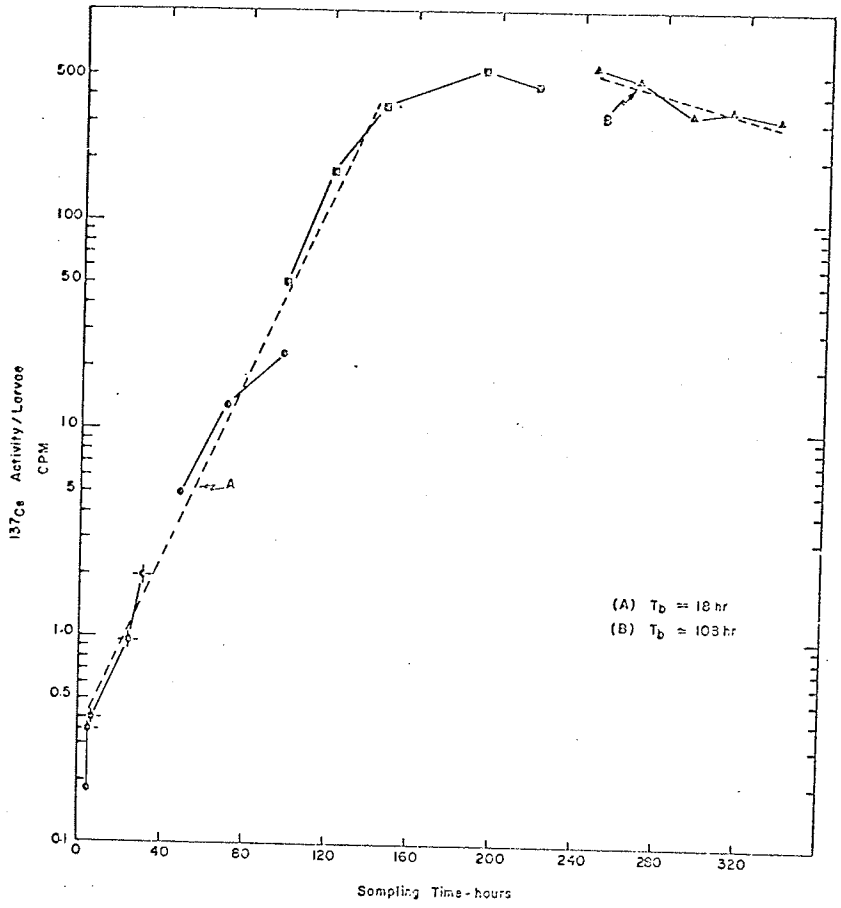

Figure 9

No. 1 Uptake by A. aegypti. Uptake of ^{137}Cs by larvae reared at 20°C and sampled at successive periods. Broken line marked A is regression line of activity of larvae sampled between 2 and 140 hrs, that marked B is regression line of larvae sampled between 240 and 330 hrs.

Coefficient of correlation (r) = 0.9926
't' test of r = 0 = 23.0768
't' test of slope (b) = 0 = 23.6666
Degrees of freedom (d.f.) = 8

The probability of this relationship occurring by chance is <0.01, and it is concluded that the slope of 'A' in Figure 9 reflects the accumulation-rate of radiocesium by all larvae sampled with the 140 hr period. Using the methods described for the computation of the biological half-life (T_b) of L. americanus, the value of the biological half-life of radiocesium uptake was calculated to be 18 hrs. Figure 9 also shows that the activity of the larvae sampled after 240 hrs decreased with time, an indication that they had ceased to feed. The broken line marked 'B' in this figure represents the least squares fit, and its statistics are:

r = -0.9307
't' test of r = 0 = 4.4069
't' test of b = 0 = -4.6666
d.f. = 8

The probability of this correlation occurring by chance is <0.02. It is tempting to conclude that the slope of 'B' (Figure 9) represents the elimination rate of radiocesium by larvae prior to molting. The value of T_b calculated from 'B' is almost 110 hrs. In the light of Crossley's suggestion (Crossley, 1963) that the elimination rate of radiocesium increases with the size of the insect, and the figure of 4.5 days previously calculated for fourth instar

L. americanus nymphs, 110 hrs (about 4.5 days) is a surprisingly large biological half-life for a late instar mosquito larva. Clearly an alternative explanation must be sought. At any given sampling time, the experimental-population will be composed of individuals which have newly molted and those who are close to ecdysis. This differentiation can be expected to increase through successive instars. The duration of the period when larvae of one instar were present was 90 hrs (Table VI), but the average duration of this period was about 48 hrs at 20°C. Thus, it is not unreasonable to expect that the samples taken of larvae contained some larvae which had recently molted from a previous instar, the instar which reached the highest radiocesium/larvae level. The activity of these larvae would tend to elevate the mean activity of the sample, causing the observed long biological half-life. Another factor which could account for the decrease in the level of radiocesium measured in larvae sampled after 240 hrs is the difference between the average amounts of the nuclide in male and female larvae. It was not possible to segregate male and female larvae for sampling, but it was a simple task to separate male and female pupae and adults. The differences between the means of male or female pupae and adults (Table VI) are not significant at the 95% confidence level. However, the grand mean of either sex, that is the mean activity of all male pupae or adults, is significantly different ($p = 0.05$) from that of female pupae or adults. Consequently, an increasing proportion of females in successive

samples of fourth instar larvae, would tend to off-set the amount of nuclide being lost as a result of biological turnover.

No. 2 Uptake by A. Aegypti Experiment

The uptake of ^{137}Cs by A. aegypti at 20°C experiment was repeated, beginning with 200 newly hatched 1st instar larvae. In this experiment, designated No. 2 Uptake by A. aegypti, emphasis was placed on determining the biological elimination rates of radiocesium by the pupal and adult instars. The results are presented in Table VII.

No. 2 Uptake experiment confirmed the previous value of 18 hrs obtained for the T_b of ^{137}Cs accumulation by larvae. As before, the logarithms of the pupal and adult radioactivities are plotted against sampling time in Figure 10. In this figure, male and female pupal activities are shown by open circles and squares, and adult male and female activities by solid circles and squares, respectively. Considering first the adults, the slope of the least-squares fitted lines represent the biological half-life which was calculated to be 40 hrs. There is no statistically significant difference between the slopes of the two adult lines, $p = 0.01$, and it is concluded that male and female adult A. aegypti eliminate ^{137}Cs at the same rate. Female A. aegypti concentrated almost twice as much ^{32}P as the male (Bruce-Chwatt and Hayward, 1956). Bugher and Taylor (1949) also reported that female A. aegypti exhibited about twice the ^{32}P and ^{89}Sr of males from the same radioactive baths. However, in all the ^{137}Cs uptake by A. aegypti

TABLE VII

No. 2 uptake of ^{137}Cs by A. aegypti. Larvae fed ground dog chow labeled with ^{137}Cs. Specific activity of food = 10^6 CPM/g. Rearing temperature = 20°C. Radiocesium reported as counts per minute (CPM) total - Beta activity. Activity and wet weights reported as mean ± one standard deviation

1 Time-hrs	2 Instar	3 Mean wt. mg	4 Mean activity CPM	5 Mean CPM/mg 4/3	6 Remarks
20	1st	-	1.1 ± 0.289	-	N = 6
43	1st	-	2.9 ± 1.08		N = 6
67	1st	-	34 ± 8.31	-	N = 6
67	2nd	-	33 ± 3.23	-	N = 6
91	2nd	0.44 ± 0.115	32 ± 10.8	72.3	N = 6
118	2nd	0.32 ± 0.071	33 ± 8.22	104.4	N = 6
118	3rd	1.2 ± 0.38	78 ± 9.01	66.6	N = 5
144	3rd	1.2 ± 0.36	363 ± 135	304.7	N = 6
144	4th	2.7 ± 0.33	542 ± 107	201.6	N = 6
168	4th	4.6 ± 0.20	594 ± 55.1	130.0	N = 6
192	4th	3.6 ± 0.89	673 ± 162	187.9	N = 4
216	4th	3.6 ± 0.39	566 ± 196	158.1	N = 6
240	4th	4.9 ± 0.31	673 ± 158	137.4	N = 6
263	4th	5.2 ± 0.34	528 ± 10.8	101.8	N = 5
281	4th	5.2 ± 0.40	428 ± 48.7	82.0	N = 6
330	4th	5.8 ± 0.69	254 ± 67.4	44.0	N = 6
216	♂ pupae	3.1 ± 0.15	305 ± 26.7	99.7	N = 6
240	♂ pupae	2.9 ± 0.28	264 ± 28.9	91.5	N = 6
263	♂ pupae	2.8 ± 0.33	262 ± 27.3	92.1	N = 4
281	♂ pupae	3.0 ± 0.56	266 ± 66.5	88.2	N = 6
330	♂ pupae	3.2 ± 0.16	211 ± 33.4	66.3	N = 6
357	pupal exuvid	0.16 ± 0.061	2.4 ± 1.37	14.9	N = 6
381	pupal exuvid	0.06 ± 0.019	5.0 ± 2.67	79.5	N = 6
216	♀ pupae	4.5 ± 0.45	584 ± 149	129.2	N = 3
240	♀ pupae	4.6 ± 0.30	613 ± 108	132.6	N = 5
263	♀ pupae	4.4 ± 0.36	545 ± 30.3	123.5	N = 6
281	♀ pupae	4.1 ± 0.49	447 ± 52.7	108.5	N = 5
330	♀ pupae	5.2 ± 0.40	207 ± 72.5	39.7	N = 5

TABLE VII Cont.d

1 Time hrs	2 Instar	3 Mean wt. mg	4 Mean activity CPM	5 Mean CPM/mg 4/3	6 Remarks
330	♂ adults	1.7 ± 0.11	209 ± 31.4	120.2	N = 6
357	♂ adults	0.58 ± 0.06	127 ± 17.5	218.0	N = 6
381	♂ adults	0.70 ± 0.12	68 ± 15.7	97.7	N = 6
405	♂ adults	1.4 ± 0.09	46 ± 12.2	32.9	N = 6
430	♂ adults	1.3 ± 0.19	33 ± 27.6	25.1	N = 4
448	♂ adults	1.2 ± 0.13	18 ± 4.30	14.9	N = 5
481	♂ adults	1.0 ± 0.14	14 ± 3.15	13.4	N = 6
500	♂ adults	1.4 ± 0.26	8.1 ± 3.46	5.9	N = 6
524	♂ adults	1.4 ± 0.08	6.8 ± 3.45	5.0	N = 6
548	♂ adults	1.4 ± 0.09	5.1 ± 1.42	3.8	N = 6
381	♂ adults	0.57 ± 0.03	122 ± 31.1	213.3	N = 6
330	♀ adults	2.9 ± 0.22	440 ± 65.4	152.4	N = 6
357	♀ adults	1.0 ± 0.09	375 ± 60.8	373.1	N = 5
381	♀ adults	0.89 ± 0.11	213 ± 101	240.6	N = 6
405	♀ adults	2.5 ± 0.14	224 ± 68.4	88.9	N = 6
430	♀ adults	2.1 ± 0.20	143 ± 46.7	68.8	N = 5
448	♀ adults	2.2 ± 0.26	115 ± 37.6	53.3	N = 6
481	♀ adults	1.8 ± 0.52	58 ± 16.2	31.6	N = 6
500	♀ adults	2.3 ± 0.26	41 ± 19.7	18.2	N = 6

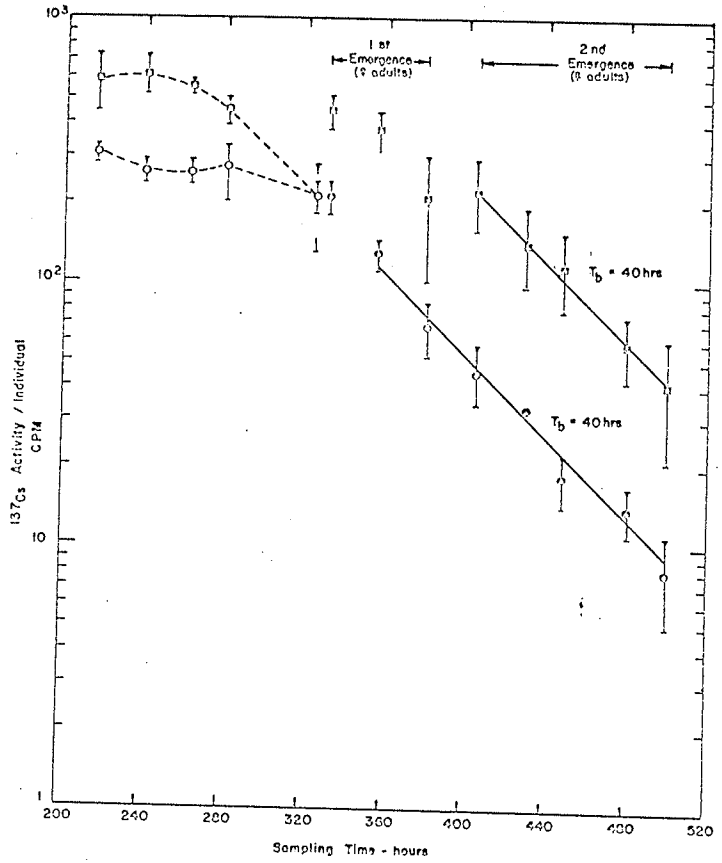

Figure 10

No. 2 Uptake by A. aegypti. Elimination of ^{137}Cs by pupae and adults reared at 20°C. Plotted points are means ± one standard deviation.

o - ♂ adults ▫ - ♀ adults
o - ♂ pupae ▫ - ♀ pupae

experiments, particularly No. 2, the differences between male and female pupae and adults are not significant at the 95% confidence level. It cannot be concluded from this experiment that the female mosquitoes accumulated more ^{137}Cs than the males. Bugher and Taylor reported from their study, that the only significant loss of ^{32}P and ^{89}Sr from adult mosquitoes resulted from egg laying (Bugher and Taylor, 1949).

The measurements reported in Table VII clearly show that the ^{137}Cs-content of the adult mosquitoes decreased by an order of magnitude during the study-interval. This decrease is reflected in the biological half-life calculated for the nuclide and is shown in Figure 10. The amount of radiocesium in the eggs laid by female mosquitoes in No. 2 Uptake experiment amounted to only a few percent of the female's body-burden. Measurement of the beta activity of fecal pellets accounted for more than 75% of the decrease in the female adult's radiocesium body-burden. The apparently different fates of ^{32}P and ^{89}Sr, compared with ^{137}Cs, is not surprising. Table I of the Committee II, International Commission on Radiological Protection (I.C.R.P.) report, lists ^{32}P and ^{90}Sr as being bone-seeking nuclides in man, and ^{137}Cs as a total-body nuclide (I.C.R.P., 1959). Phosphorous and strontium being "target-organ" seekers, one could expect them to be retained longer in adult mosquitoes, than a radionuclide such as ^{137}Cs which is distributed throughout the animal's body. More than 40% of the ^{32}P or ^{89}Sr accumulated by adult A. aegypti is deposited in

the legs (Bugher and Taylor, 1949), a finding which supports the suggestion that the biological half-life of radiocesium in the adult mosquito is shorter than that of ^{32}P or ^{89}Sr, because ^{137}Cs is not concentrated by any particular organ. Attempts to demonstrate localization of radiocesium in A. aegypti larvae and adults by autoradiography were unsuccessful. What is remarkable is the magnitude of the 40 hr-T_b value obtained for the adults. As cited previously, Crossley (1963) suggested that the biological half-life of radiocesium increases with the size of the insect. The 40 hr half-life measured for adult A. aegypti places them in the same class as grasshoppers (Crossley and Pryor, 1960; Crossley and Schnell, 1961), insects much larger than mosquitoes but, not aquatic. When discussing radiocesium elimination by L. americanus, it was pointed out that the value of T_b obtained for nymphal stages was smaller than that measured for adults. Similarly, the uptake rates of early instar mosquitoes, Figure 9, are less than those of 4th instar and adult mosquitoes (Figures 9 and 10).

The radioactivities of the pupae given in Table VII suggest that some of the pupal ^{137}Cs body-burden may have escaped through the exuvia. A regression analysis of the pertinent measurements, plotted in Figure 10, gave the following statistics:

	♂ pupae	♀ pupae
Correlation coefficient 'r'	-0.9417	-0.9207
t-test of r = 0	4.8454	4.0847
t-test of b = 0	-6.000	-4.1000
standard deviation of y on s ($S_{y,x}$)-	0.0223	0.0877
standard deviation of b (S_b)	0.002	0.0010
degrees of freedom (d. of f.)	3	3

Some of the pupae's radiocesium body-burden may have passed through the exuvia into the surrounding water, in which the pupal were placed to emerge. Samples of this water were counted and found to have count-rates significantly higher than those of comparable samples of distilled water. This finding can be accounted for by radiocesium de-sorbing from the exuvia. The method employed could not differentiate between ^{137}Cs which passed through the exuvia, and that de-sorbed from the exterior surface of the pupa. The former explanation, in the light of reports by Hasset and Jenkins (1951) and other workers, is not too probable.

No. 3 Uptake by A. aegypti Experiment

No. 3 Uptake of radiocesium experiment was carried out at 25°C, five degrees higher than Nos. 1 and 2. The results are recorded in Table VIII, and plotted in Figure 11. Reichle (1967) for example, has shown radiocesium-turnover to be a function of metabolic activity. One could expect, therefore, that the biological elimination rate of ^{137}Cs at 25°C would be greater than it is at 20°C. This increase in turnover-time would be reflected

TABLE VIII

No. 3 uptake of ^{137}Cs by A. aegypti. Larvae fed ground dog chow labeled with ^{137}Cs. Specific activity of food = 10^6 CPM/g. Rearing temperature - 25°C. Radiocesium reported as counts per minute (CPM) total - Beta activity. Activity and dry (dried overnight in a $CaCl_2$ dessicator) weights reported as mean ± one standard deviation

1 Time-hrs	2 Instar	3 Mean wt. mg	4 Mean Activity CPM	5 Mean CPM/mg 4/3	6 Remarks
24	1st	0.05 ± 0.02	5.9 ± 2.37	118.0	N = 12
48	2nd	0.08 ± 0.01	13 ± 4.13	162.5	N = 12
72	2nd	0.53 ± 0.15	11 ± 5.24	20.8	N = 9
72	3rd	0.27 ± 0.05	78 ± 22.5	288.8	N = 12
96	3rd	0.50 ± 0.10	285 ± 58.0	570.0	N = 11
96	4th	0.79 ± 0.05	321 ± 31.7	406.3	N = 6
120	4th	1.2 ± 0.13	373 ± 84.4	310.8	N = 12
144	4th	1.3 ± 0.14	313 ± 41.3	240.8	N = 6
120	♂ pupae	0.68 ± 0.05	137 ± 27.0	201.5	N = 12
144	♀ pupae	1.2 ± 0.07	283 ± 59.3	235.8	N = 12
216	pupal exuvia	0.06 ± 0.01	0.8 ± 0.44	13.3	N = 12
216	♂ adults	0.53 ± 0.03	31 ± 7.10	58.5	N = 11
288	♂ adults	0.37 ± 0.04	19 ± 10.4	51.4	N = 8
216	♀ adults	0.89 ± 0.07	110 ± 32.3	123.6	N = 12
288	♀ adults	0.64 ± 0.10	67 ± 62.9	104.7	N = 11

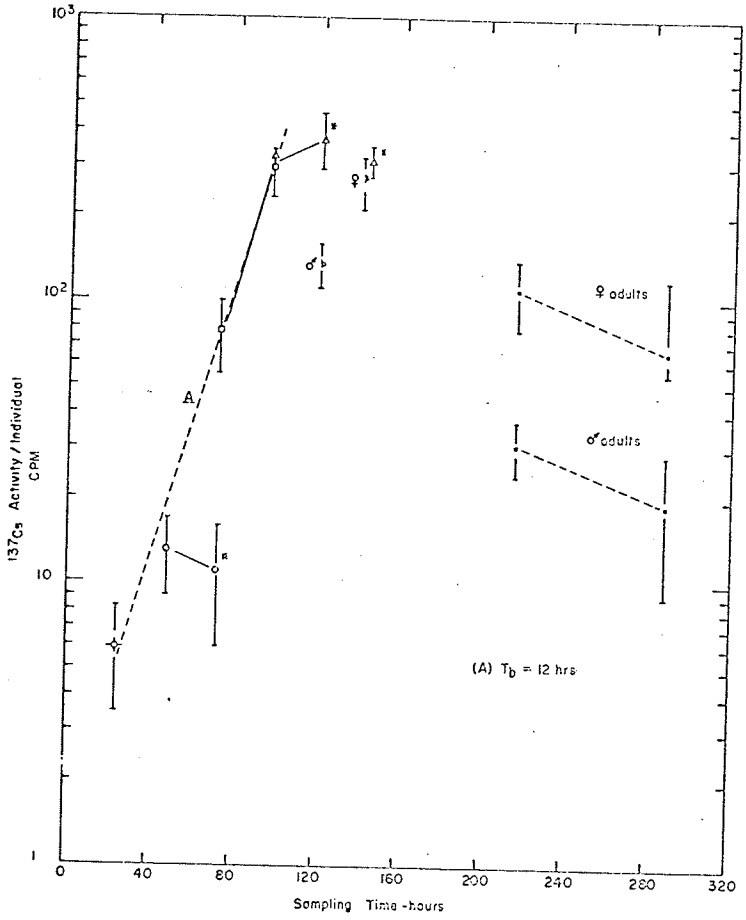

Figure 11

No. 3 Uptake by A. aegypti. Uptake of ^{137}Cs by larvae reared at 25°C. Plotted points are means ± one standard deviation. Broken line marked A is regression line of activity on time omitting points marked *.

⊹ - 1st instar 0 - 2nd instar □ - 3rd instar
△ - 4th instar p - pupae
T_b = biological half-life

in a lower value for T_b. Examination of the broken-line marked 'A' in Figure 11, shows that the rate of uptake of radiocesium was faster at 25°C, than it was at 20°C. The value T_b = 12 hrs, is significantly less (p = 0.05) than T_b = 18 hrs, determined for similar larval stages reared at 20°C (Figure 9). Line 'A' in Figure 11 was fitted by the method of least squares, omitting the points marked *. The statistics of the fit obtained are:

r	- 0.9925
b	- 0.0248
t-test of r = 0	- 14.0356
t-test of b = 0	- 14.5882
$S_{y,x}$	- 0.1100
S_b	- 0.0017
d. of f.	- 3

For some reason, for which there is no obvious explanation, few of the adults in No. 3 Uptake experiment survived long enough to measure their ^{137}Cs elimination rate. The amounts of the nuclide measured in the males and females did not differ significantly, p = 0.05.

Uptake of ^{137}Cs by A. aegypti Pupae

It has been pointed out that the radiocesium activity of A. aegypti pupae decreased with time (Table VII). The observed decrease could be accounted for by de-sorption of the nuclide from the pupal exuvia, or it might be the result of ^{137}Cs escaping from the pupa through the exuvia.

To test these two hypotheses, a group of 100 24 hr old A. aegypti pupae were placed in distilled water (controls), a second group was placed in the same volume of distilled water containing 8×10^3 cpm/ml carrier-free ^{137}Cs for 24 hrs, and a third group in the same concentration of radiocesium for 72 hrs. All groups were maintained at 20°C; the pupae had pupated from larvae reared at the same temperature. Following the treatments, all groups of pupae were washed three times in equal volumes of distilled water, then each placed in 100 ml of water to emerge. Adults were captured and total-beta counted within 12 hrs of emerging, to avoid loss of ^{137}Cs by excretion. The exuvia and samples of the emergence-water, were also counted.

The results of this experiment can be briefly summarized. None of the male or female adults which emerged from pupae immersed in the radiocesium solution were significantly more radioactive than the controls, (Students 't' test, $p = 0.05$). A material balance, based on the activity of the ^{137}Cs solutions before and after pupal exposure, the pupae after exposure, the emergence-water, and the activity of exuvia, showed that de-sorption of ^{137}Cs from the pupa could account for the decrease in pupal activity observed in No. 2 Uptake experiment.

Uptake and Elimination of ^{137}Cs by Aedes atropalpus (Coquillett)

A series of experiments to measure uptake and elimination rates of radiocesium by Aedes atropalpus, a mosquito species native to Canada, were carried out under conditions

comparable to those employed for A. aegypti measurements. The first of this series, No. 1 Uptake by A. atropalpus, employed a rearing temperature of 20°C. The results of the experiment are tabulated in Table IX, from which it will be immediately apparent that the mortality was quite high. For the purpose of T_b measurement, the measurements are valueless. High mortality and poor weight-gain, compared to A. atropalpus fed non-labeled food, suggested the larvae perhaps suffered radiation damage as a result of the specific activity of their food, 1×10^7 cpm/g, giving an integrated radiation dose of about 2 rad/larva/day. No. 1 A. atropalpus Uptake experiment was repeated (No. 2 Uptake experiment) at a rearing temperature of 25°C (Table X). Few of these larvae survived beyond the 3rd instar. The weight-gain suggested that the larvae may have fed poorly. However, the measurements reported in Table X do indicate that the accumulation rate of 1st, 2nd and 3rd instar A. atropalpus was about 12 hrs, comparable to that of A. aegypti reared at 25°C.

A new batch of ^{137}Cs labeled A. atropalpus food was prepared, specific activity about 10^3 cpm/g, and used to feed the larvae in No. 3 Uptake experiment. The weight-gain of these larvae (Table XI) compared favourably with that of A. atropalpus fed non-labeled food. Unfortunately, the activity of the radiocesium label was too low, and no useful results were obtained. Therefore a fourth uptake experiment employing another batch of labeled food, specific activity 1.25×10^4 cpm/g, and a rearing

TABLE IX

No. 1 Uptake of ^{137}Cs by A. atropalpus. Larvae fed fish rearing food labeled with ^{137}Cs. Specific activity of food = 10^7 CPM/g. Rearing temperature = 20°C. Radiocesium reported as counts per minute (CPM) total - Beta activity. Activity and dry (dried overnight in a $CaCl_2$ dessicator) weights reported as mean ± one standard deviation

1 Time-hrs	2 Instar	3 Mean wt. mg	4 Mean activity CPM	5 Mean CPM/g 4/3	6 Remarks
24	2nd	0.04 ± 0.008	8.0 ± 2.24	269.5	N = 9
47	2nd	lost	46 ± 14.3	-	N = 12
69	2nd	lost	46 ± 10.8	-	N = 10
97	2nd	lost	4.2 ± 4.88	-	N = 6
69	3rd	lost	78 ± 38.6	-	N = 12
97	3rd	lost	46 ± 25.7	-	N = 11
117	3rd	0.21 ± 0.042	370 ± 82.5	1770	N = 11
144	3rd	0.11 ± 0.041	381 ± 112	3628	N = 5
235	3rd	0.16	642	4111	N = 1
235	4th	0.67	1082	1615	N = 1
235	♂ pupae	0.65 ± 0.05	732 ± 142	1134	N = 2

TABLE X

No. 2 uptake of ^{137}Cs by A. atropalpus. Larvae fed fish rearing food labeled with ^{137}Cs. Specific activity of food = 10^7 CPM/g. Rearing temperature = 25°C. Radiocesium reported as counts per minute (CPM) total - Beta activity. Activity and dry (dried overnight in a $CaCl_2$ dessicator) weights reported as mean ± one standard deviation

1 Time hrs	2 Instar	3 Mean wt. mg	4 Mean activity CPM	5 Mean CPM/g 4/3	6 Remarks
24	1st	0.012 ± 0.005	12.3 ± 5.27	1025	N = 10
46	2nd	0.036 ± 0.006	57 ± 15.5	1580	N = 11
70	2nd	0.047 ± 0.008	60 ± 21.9	1280	N = 12
70	3rd	0.084 ± 0.013	193 ± 51.8	2300	N = 9
92	3rd	0.17 ± 0.013	488 ± 67.6	2880	N = 6
142	4th				

TABLE XI

Total - Beta activity of A. atropalpus fed labeled fish rearing food, specific activity $<10^3$ CPM/g. Rearing temperature = 25°C. Activity in counts per minute (CPM) and day (dried overnight in a $CaCl_2$ dessicator) weights reported as mean ± one standard deviation

1 Time-hrs	2 Instar	3 Mean wt mg	4 Mean activity CPM	5 Mean CPM/mg 4/3	6 Remarks
24	1st	-	0.22 ± 0.344	-	N = 6
49	2nd	0.05 ± 0.020	0.13 ± 0.117	2.6	N = 12
49	3rd	0.12 ± 0.019	0.45 ± 0.411	3.8	N = 6
72	3rd	0.13 ± 0.031	0.41 ± 0.536	3.2	N = 6
120	4th	0.90 ± 0.239	0.38 ± 0.199	0.42	N = 9
143	4th	1.24 ± 0.275	0.38 ± 0.147	0.31	N = 6
120	♂ pupae	0.65 ± 0.053	0.16 ± 0.128	0.25	N = 12
120	♀ pupae	1.33 ± 0.104	0.43 ± 0.360	0.32	N = 12
143	♂ pupae	0.63 ± 0.035	0.38 ± 0.172	0.60	N = 6
143	♀ pupae	1.53 ± 0.064	0.21 ± 0.276	0.14	N = 2
168	♂ adults	0.47 ± 0.038	0.34 ± 0.275	0.72	N = 17
193	♂ adults	0.41 ± 0.037	0.48 ± 0.423	1.2	N = 11
217	♂ adults	0.33 ± 0.026	0.34 ± 0.562	1.0	N = 9
241	♂ adults	0.29 ± 0.040	0.19 ± 0.215	0.66	N = 7
168	♀ adults	1.11 ± 0.089	0.37 ± 0.181	0.33	N = 12
193	♀ adults	0.96 ± 0.096	0.33 ± 0.450	0.34	N = 11
217	♀ adults	0.86 ± 0.092	0.16 ± 0.171	0.19	N = 6

temperature of 26°C was set up. The results of this experiment were also inconclusive, the activity of the larvae being about the same as in the other uptake experiments. The effect of potassium concentration on radiocesium uptake, to be described, suggests the failure of A. atropalpus to accumulate radiocesium may have been the result of the amount of potassium in the food. Analysis of the rearing medium showed that sufficient potassium leached from the A. atropalpus food in 48 hrs to produce a concentration in the rearing medium of about 0.002 M. It is postulated that the apparent failure of A. atropalpus to accumulate ^{137}Cs was the result of suppression of uptake by potassium, and the adequacy of fish food as a diet for this species.

CHAPTER VI

EFFECT OF POTASSIUM CONCENTRATION ON ^{137}Cs UPTAKE

INTRODUCTION

In the course of the radiocesium uptake by A. aegypti experiments the question arose: Can an ion chemically similar to cesium affect the accumulation of that nuclide by the mosquito, in a manner comparable to the suppression of strontium uptake by calcium? Potassium is an element of considerable importance to the invertebrate and vertebrate body. Because of its similarity to potassium, cesium might be expected to behave biologically in a similar manner. The ecological relationships of potassium and cesium have been extensively reviewed by Davis (1963), therefore only the more outstanding aspects of the question - the inter-relationship of potassium and cesium - will be discussed. The topic is still very much one for debate.

Handley and Overstreet (1961) demonstrated that in low external concentrations of cesium, potassium significantly affected the uptake of ^{137}Cs by barley roots. Uptake experiments using millet, oats, and sweet clover growing in nutrient solutions have suggested discrimination by these plants against cesium, in favor of potassium (Menzell and Heald, 1955). Depression of cesium uptake in the presence of potassium has also been demonstrated for the alga Chlorella (Morgan and Meyers, 1953), and for yeast cells (Rothstein, 1955). Cline reported that the uptake and translocation of ^{137}Cs

and K^+ were markedly influenced by variations in potassium concentration of the medium in which red kidney beans were growing. However, he concluded that the behaviour of cesium and potassium in plants was largely independent of each other (Cline, 1962). Evans and Dekker have studied the uptake of radiocesium by oats and alfalfa in the laboratory and in the greenhouse. They found accumulation of this radionuclide to be related to the degree that it was fixed in the soil. Plants grown on soils with a low concentration of available potassium, accumulated the most radiocesium (Evans and Dekker, 1966). In contrast, it has been reported that the suppression of ^{137}Cs-uptake by potassium is quite small, and generally of little consequence (Williams and Swanson, 1958; Williams, 1960, for example). Scott (1954) has reported that marine algae cells attempt to utilize cesium to fulfill potassium deficiency. Metabolic similarities between cesium and potassium in animals have been recognized since 1882, when Ringer compared the effects of cesium, potassium, and rubidium on the activity of the isolated frog heart (Ringer, 1882). Loeb observed that potassium was an essential constituent of water for the development of sea urchin eggs, and that these requirements could be provided for by the replacement of potassium with cesium (Loeb, 1921). The addition of supplementary potassium to the diets of mammals has been shown to reduce retention of cesium in their tissues (Mraz, 1959; Wasserman and Comar, 1961). Measurements of ^{137}Cs and potassium in the edible tissue of species of freshwater fish caught in the Arctic Ocean and certain lakes in south Finland, showed

a tendency towards an inverse relationship (Hüsänen and Miettinen, 1963). Precise comparison was difficult because the levels of fall-out fission products in the lakes were different. In general, the opinion of investigators concerned with radiocesium accumulation in fish is summed up by Gustafson et al. (1966) - "Cesium-137 concentration present in fish depends primarily on three factors: (1) the concentration of the radionuclide in the water environment; (2) the accompanying level of potassium (ratio of cesium-137: potassium); (3) the position of the fish in question in the aquatic food chain". Little comparable work on invertebrates has been reported, however Gustafson et al. postulate provided a satisfactory working hypothesis for insects, particularly an investigation of potassium effects on the accumulation of ^{137}Cs by A. aegypti.

METHODS

The only departure from the mosquito methods which have been described, was the presence of various concentrations of potassium chloride in the rearing medium. Larvae were fed ^{137}Cs labeled Gaine's dog-meal as before, and reared at 20°C and 25°C. This series of experiments were set up as a completely randomized design. The various instars were sampled daily. Groups of 100 newly emerged 1st instar A. aegypti larvae were reared in distilled water, and in pans containing 0.005, 0.01, 0.025, 0.05, 0.075, 0.10 and 0.25 molar solutions of potassium chloride in distilled water.

RESULTS AND DISCUSSION

The results of these experiments are presented in Tables XII and XIII. All larvae reared in 0.25 M potassium chloride solutions at 20°C and 25°C died within 24 hrs. It will be seen from these tables that the difference between the amount of radiocesium accumulated by the controls, and the amounts taken up by larvae in the various potassium concentrations, increased with successive instars. Any effect that potassium may have exerted on ^{137}Cs uptake at 25°C is not pronounced before the third instar.

The apparent differences between the amounts of radiocesium accumulated by male and female pupae and adults are not significant at the 95% confidence level. The variation between radiocesium concentration of larvae in each potassium concentration and that of ^{137}Cs uptake with temperature at a given potassium concentration, were examined by the analysis of variance method (Bailey, 1959). The results of this analysis permit the following conclusions about the measurements reported in Tables XII and XIII: (1) The differences between the amounts of ^{137}Cs accumulated by the controls and the activities of larvae, pupae, and adults reared at 20°C or 25°C in the various potassium concentrations, are statistically significant ($p = 0.05$). (2) The apparently greater suppression of ^{137}Cs uptake by potassium at 25°C, compared to 20°C, is also statistically significant ($p = 0.05$). (3) All the potassium concentrations examined suppressed ^{137}Cs uptake to the same degree. Significant differences between individual potassium concentrations, where they occur, were random.

TABLE XII

Effect of potassium concentration on uptake of ^{137}Cs by A. aegypti reared at 20°C. Larvae fed dog chow labeled with ^{137}Cs. Specific activity of food = 10^6CPM/g. Activity and wet weights reported as mean ± one standard deviation

1 Time-hrs	2 Instar	3 Mean wt. mg	4 Mean Activity CPM	5 Mean Activity/mg 4/3	6 Remarks
CONTROLS					
24	1st	-	0.85 ± 0.73	-	N = 6
49	1st	-	4.2 ± 1.28	-	N = 6
70	1st	-	8.6 ± 2.18	-	N = 6
70	2nd	-	2.4 ± 1.17	-	N = 3
97	2nd	-	3.7 ± 1.47	-	N = 6
119	2nd	-	36 ± 4.9	-	N = 6
119	3rd	0.23 ± 0.05	50 ± 20.3	218	N = 6
167	4th	0.66 ± 0.08	292 ± 24.0	442	N = 6
191	4th	1.01 ± 0.07	477 ± 49.0	472	N = 5
237	4th	1.24 ± 0.31	349 ± 39.4	2812	N = 3
237	♂ pupa	0.78 ± 0.04	145 ± 13.6	186	N = 6
333-357	♀ adults	1.23 ± 0.03	224 ± 14.5	182	N = 3
333-357	♂ adults	0.75 ± 0.04	99 ± 21.1	131	N = 6
381	exuvia	0.05 ± 0.008	0.93 ± 0.78	18.6	N = 6
0.005M					
24	1st	-	0.72 ± 0.36	-	N = 6
49	1st	-	2.9 ± 0.85	-	N = 6
70	1st	-	3.9 ± 1.136	-	N = 6
70	2nd	-	0.55 ± 0.44	-	N = 6
97	2nd	-	1.5 ± 0.60	-	N = 6
97	3rd	-	4.8 ± 3.58	-	N = 6
119	3rd	0.24 ± 0.04	25 ± 5.34	104	N = 6

TABLE XII Cont.d

1 Time hrs	2 Instar	3 Mean wt. mg	4 Mean Activity CPM	5 Mean Activity/mg 4/3	6 Remarks
167	4th	0.85 ± 0.15	118 ± 17.3	138	N = 6
191	4th	1.12 ± 0.13	89 ± 18.3	80	N = 6
237	♂ pupa	0.77 ± 0.03	77 ± 8.96	99	N = 6
261	♀ pupa	1.57 ± 0.10	123 ± 15.50	123	N = 3
309	♂ adults	0.82 ± 0.05	50 ± 12.34	61	N = 6
333-381	♀ adults	1.25 ± 0.06	92 ± 16.07	74	N = 5
381	exuvia	0.05 ± 0.02	0.98 ± 0.762	20	N = 4
0.01M					
24	1st	-	0.72 ± 0.32	-	N = 6
49	1st	-	3.2 ± 1.03	-	N = 6
70	1st	-	2.4 ± 1.17	-	N = 6
70	2nd	-	0.54 ± 0.76	-	N = 6
97	2nd	-	1.6 ± 0.45	-	N = 6
97	3rd	-	3.0 ± 2.98	-	N = 6
120	3rd	0.26 ± 0.05	23 ± 4.43	89	N = 6
168	3rd	0.49 ± 0.13	62 ± 18.8	104	N = 6
168	4th	0.90 ± 01.3	115 ± 28.1	127	N = 6
191	4th	1.32 ± 0.13	103 ± 26.5	78	N = 6
238	4th	1.47 ± 0.13	149 ± 47.2	102	N = 3
238	♂ pupa	0.82 ± 0.07	64 ± 22.0	77	N = 6
309	♂ adults	0.78 ± 0.06	26 ± 13.5	33	N = 6
333-381	♀ adults	1.29 ± 0.10	93 ± 13.1	72	N = 6
0.025M					
24	1st	-	0.5 ± 0.78	-	N = 6
49	1st	-	3.5 ± 0.80	-	N = 6
70	1st	-	4.1 ± 0.72	-	N = 6
70	2nd	-	0.43 ± 0.30	-	N = 6
98	2nd	-	2.9 ± 2.9	-	N = 6

TABLE XII Cont.d

1 Time-hrs	2 Instar	3 Mean wt. mg	4 Mean Activity CPM	5 Mean Activity/mg 4/3	6 Remarks
98	3rd	-	3.7 ± 2.20	-	N = 6
120	3rd	0.26 ± 0.04	20 ± 4.28	77	N = 6
168	3rd	0.38 ± 0.18	39 ± 22.8	103	N = 6
168	4th	0.87 ± 0.18	96 ± 9.36	110	N = 6
192	4th	1.17 ± 0.13	76 ± 14.8	65	N = 6
239	4th	1.16 ± 0.36	120 ± 44.8	103	N = 5
239	♂ pupa	0.81 ± 0.10	59 ± 11.8	73	N = 6
309	♂ adults	0.83 ± 0.05	37 ± 7.60	44	N = 6
333-381	♀ adults	1.25 ± 0.09	82 ± 18.3	65	N = 6
381	exuvia	0.05 ± 0.01	1.5 ± 1.18	29	N = 6
0.05M					
24	1st	-	0.92 ± 0.623	-	N = 6
49	1st	-	4.3 ± 2.46	-	N = 6
69	1st	-	11 ± 2.54	-	N = 6
69	2nd	-	0.03 ± 0.049	-	N = 6
99	2nd	-	1.4 ± 0.84	-	N = 6
99	3rd	-	1.0 ± 0.64	-	N = 6
120	3rd	0.21 ± 0.05	15 ± 3.91	70	N = 6
168	3rd	0.41 ± 0.12	32 ± 10.1	78	N = 6
168	4th	0.75 ± 0.08	77 ± 13.9	103	N = 6
192	4th	1.08 ± 0.13	49 ± 11.1	46	N = 6
240	4th	1.35 ± 0.06	61 ± 9.33	45	N = 2
240	♂ pupa	0.83 ± 0.05	35 ± 6.48	42	N = 6
262	♀ pupa	2.34 ± 1.33	60 ± 10.2	25	N = 3
309	♂ adults	0.66 ± 0.06	23 ± 5.43	35	N = 6
357-381	♀ adults	1.12 ± 0.07	41 ± 4.33	36	N = 3
381	exuvia	0.06 ± 0.01	0.22 ± 0.133	3.7	N = 6

TABLE XII Cont'd.

1 Time-hrs	2 Instar	3 Mean wt. mg	4 Mean Activity CPM	5 Mean Activity/mg 4/3	6 Remarks
0.075M					
24	1st	-	0.64 ± 0.592	-	N = 6
49	1st	-	10 ± 4.81	-	N = 6
69	1st	-	8.8 ± 2.54	-	N = 6
69	2nd	-	0.99 ± 0.726	-	N = 6
99	2nd	-	0.17 ± 0.208	-	N = 6
99	3rd	-	4.6 ± 6.93	-	N = 6
120	3rd	0.24 ± 0.03	11 ± 3.64	44	N = 6
168	3rd	0.31 ± 0.12	19 ± 8.79	62	N = 6
168	4th	0.77 ± 0.09	44 ± 4.83	57	N = 6
192	4th	1.21 ± 0.22	40 ± 10.1	33	N = 6
240	4th	1.38 ± 0.19	52 ± 5.22	38	N = 5
240	♂ pupa	0.84 ± 0.05	32 ± 7.17	38	N = 6
309	♂ adults	0.69 ± 0.05	17 ± 2.83	25	N = 6
333-381	♀ adults	1.11 ± 0.07	39 ± 4.39	35	N = 6
381	exuvia	0.05 ± 0.013	0.19 ± 0.189	38	N = 5
0.1M					
24	1st	-	0.60 ± 0.445	-	N = 5
49	1st	-	23 ± 17.9	-	N = 6
69	1st	-	7.4 ± 1.02	-	N = 6
69	2nd	-	0.17 ± 0.142	-	N = 6
99	2nd	-	0.29 ± 0.227	-	N = 6
120	2nd	-	22 ± 3.79	-	N = 6
99	3rd	-	1.2 ± 0.378	-	N = 6
120	3rd	0.14 ± 0.03	8.7 ± 2.45	62	N = 6
168	3rd	0.26 ± 0.06	21 ± 7.44	81	N = 5
241	3rd	0.60 ± 0.15	31 ± 5.02	52	N = 6
260	4th	1.09 ± 0.22	63 ± 16.7	57	N = 6

TABLE XII Cont'd.

1 Time-hrs	2 Instar	3 Mean wt. mg	4 Mean Activity CPM	5 Mean Activity/mg 4/3	6 Remarks
357-381	♂ adults	0.55 ± 0.03	38 ± 9.70	70	N = 3
381	♀ adults	0.92 ± 0.01	31 ± 0.707	34	N = 2
381	exuvia	0.04 ± 0.02	1.2 ± 1.45	30	N = 5

All larvae died within 24 hrs - 0.25M

TABLE XIII

Effect of potassium concentration on uptake of ^{137}Cs by A. aegypti reared at 25°C. Larvae fed dog chow labeled with ^{137}Cs. Specific activity of food = 10^6CPM/g. Activity and wet weights reported as mean ± one standard deviation

1 Time-hrs	2 Instar	3 Mean wt. mg	4 Mean Activity CPM	5 Mean Activity/mg 4/3	6 Remarks
CONTROLS					
24	1st	0.018 ± 0.008	0.97 ± 0.461	54	N = 6
48	2nd	0.06 ± 0.010	15 ± 5.76	266	N = 6
97	3rd	0.49 ± 0.15	299 ± 117	611	N = 5
141	3rd	0.36 ± 0.15	137 ± 54.0	380	N = 3
165	4th	0.72 ± 0.11	201 ± 69.5	278	N = 2
165	♂ pupa	0.58 ± 0.13	269 ± 112	463	N = 4
165	♀ pupa	0.58	367	632	N = 1
0.005M					
24	1st	0.02 ± 0.006	2.5 ± 1.55	123	N = 5
48	2nd	0.07 ± 0.03	7.9 ± 2.14	112	N = 6
69	2nd	0.27 ± 0.05	11 ± 6.47	42	N = 6
69	3rd	0.44 ± 0.03	23 ± 7.47	52	N = 6
98	3rd	0.58 ± 0.13	39 ± 7.97	66	N = 6
98	4th	0.76 ± 0.15	56 ± 8.74	74	N = 6
117	4th	0.88 ± 0.33	42 ± 11.8	47	N = 6
165	4th	0.75 ± 0.41	48 ± 15.0	64	N = 2
141	♂ pupa	0.47 ± 0.03	29 ± 1.81	62	N = 5
141	♀ pupa	0.88 ± 0.08	41 ± 5.49	46	N = 3
165	pupal exuvia	0.043 ± 0.015	3.3 ± 3.61	78	N = 3
214	pupal exuvia	0.040 ± 0.009	0.2 ± 0.187	5	N = 6
214	♂ adults	0.24 ± 0.035	4.3 ± 1.20	18	N = 2
214	♀ adults	0.47 ± 0.130	24 ± 10.2	51	N = 5

TABLE XIII Cont'd.

1 Time-hrs	2 Instar	3 Mean wt. mg	4 Mean Activity CPM	5 Mean Activity/mg 4/3	6 Remarks
0.01M					
24	1st	0.03 ± 0.008	0.50 ± 0.644	15	N = 6
49	2nd	0.10 ± 0.01	3.7 ± 0.949	37	N = 6
69	2nd	0.22 ± 0.03	9.7 ± 1.98	44	N = 6
98	3rd	0.62 ± 0.11	33 ± 8.69	53	N = 6
117	3rd	0.74 ± 0.03	35 ± 7.86	48	N = 6
117	4th	1.12 ± 0.30	74 ± 19.6	66	N = 6
141	♂ pupa	0.99 ± 0.17	52 ± 11.8	52	N = 6
165	♀ pupa	1.15 ± 0.13	69 ± 4.46	69	N = 2
165	pupal exuvia	0.07 ± 0.02	0.92 ± 0.311	13	N = 2
213	pupal exuvia	0.07 ± 0.01	0.72 ± 0.653	10	N = 6
213	♂ adults	0.52 ± 0.10	5.4 ± 2.32	10	N = 4
213	♀ adults	0.79 ± 0.10	11 ± 4.47	14	N = 5
0.025M					
24	1st	0.026 ± 0.005	0.20 ± 0.244	7.7	N = 5
49	2nd	0.06 ± 0.02	3.5 ± 0.632	58	N = 6
69	2nd	0.13 ± 0.03	5.6 ± 0.976	43	N = 6
98	3rd	0.52 ± 0.07	29 ± 8.73	55	N = 6
117	3rd	0.22 ± 0.13	10 ± 5.51	47	N = 3
98	4th	0.71 ± 0.10	37 ± 8.09	52	N = 4
141	4th	1.11 ± 0.21	38 ± 5.71	35	N = 4
141	♂ pupa	0.71 ± 0.08	20 ± 1.31	28	N = 5
141	♀ pupa	1.23	39.4	32	N = 1
0.05M					
24	1st	0.62 ± 0.007	0.01 ± 0.016	0.5	N = 6

TABLE XIII Cont'd.

1 Time-hrs	2 Instar	3 Mean wt. mg	4 Mean Activity CPM	5 Mean Activity/mg 4/3	6 Remarks
49	2nd	0.11 ± 0.02	3.3 ± 1.10	30	N = 6
69	2nd	0.17 ± 0.02	6.0 ± 1.31	35	N = 6
69	3rd	0.33 ± 0.05	12 ± 2.42	38	N = 6
98	3rd	0.56 ± 0.06	15 ± 1.55	26	N = 6
141	3rd	0.58 ± 0.19	13 ± 2.33	22	N = 3
98	4th	0.95 ± 0.14	23 ± 3.99	24	N = 6
117	4th	0.98 ± 0.17	32 ± 7.05	33	N = 6
141	4th	1.27	40	32	N = 1
141	♂ pupa	0.63 ± 0.07	17 ± 1.87	27	N = 6
141	♀ pupa	1.00 ± 0.13	29 ± 5.21	29	N = 6
213	pupal exuvia	0.08 ± 0.02	0.20 ± 0.248	2.5	N = 6
213	♂ adults	0.43 ± 0.070	2.7 ± 1.54	6.2	N = 6
213	♀ adults	0.82 ± 0.100	11 ± 6.28	13	N = 6
0.075M					
24	1st	0.02 ± 0.008	0.20 ± 0.243	10	N = 6
49	2nd	0.08 ± 0.009	4.9 ± 2.24	61	N = 6
69	2nd	0.14 ± 0.02	5.1 ± 0.757		N = 5
98	3rd	0.51 ± 0.06	14 ± 2.20	27	N = 6
117	3rd	0.34 ± 0.20	12 ± 7.06	36	N = 3
98	4th	0.78 ± 0.06	19 ± 1.66	24	N = 6
117	4th	1.17 ± 0.11	38 ± 4.92	32	N = 6
141	4th	1.01 ± 0.02	33 ± 2.33	33	N = 2
141	♂ pupa	0.65 ± 0.0	18 ± 1.84	28	N = 2
141	♀ pupa	0.90 ± 0.15	29 ± 6.52	32	N = 5
213	pupal exuvia	0.09 ± 0.04	0.08 ± 0.147	0.89	N = 5
213	♀ adults	0.74 ± 0.10	8.4 ± 3.85	11	N = 5

TABLE XIII Cont'd.

1 Time-hrs	2 Instar	3 Mean wt. mg	4 Mean Activity CPM	5 Mean Activity/mg 4/3	6 Remarks
0.10M					
24	1st	0.03 ± 0.008	0.18 ± 0.125	5.7	N = 4
50	2nd	0.12 ± 0.030	1.9 ± 0.461	16	N = 6
69	2nd	0.18 ± 0.020	4.0 ± 1.57	22	N = 6
98	3rd	0.58 ± 0.08	14 ± 3.09	25	N = 6
117	3rd	0.61 ± 0.04	16 ± 2.05	26	N = 3
98	4th	0.75 ± 0.06	21 ± 4.46	28	N = 4
117	4th	0.92 ± 0.22	23 ± 6.65	25	N = 6
141	4th	1.09 ± 0.29	19 ± 8.47	17	N = 3
141	♂ pupa	0.68 ± 0.06	13 ± 2.14	19	N = 3
141	♀ pupa	0.83	21	26	N = 1
165	♀ pupa	0.94 ± 0.08	16 ± 0.707	17	N = 2
189	♀ pupa	1.02 ± 0.06	25 ± 8.98	25	N = 2
213	pupal exuvia	0.09 ± 0.01	0.23 ± 0.247	2.6	N = 3
213	♂ adults	0.48	1.2	2.3	N = 1
213	♀ adults	0.88 ± 0.01	11 ± 4.03	11	N = 2

The degree of suppression of radiocesium uptake by potassium at a given temperature appeared to be constant, and independent of the concentration of the latter ion. This finding could be a manifestation of a saturation effect. That is, all available papillary membrane transport sites for K^+ were occupied at the concentrations examined, effectively restricting diffusion of cesium ions. To test this hypothesis, a radiocesium uptake experiment was run at 25°C, following the same procedure, and using concentrations of potassium chloride in distilled water 0.001 M, 0.0025M, and 0.005M, and controls. The results of this experiment are given in Table XIV. Once again, the differences between male and female pupae and adults were not significant at the 95% confidence level. It will be noted from this table that the mean activity of the larval and pupal exuviae from the 0.001 M potassium concentration is greater than that of the controls. This difference is significant ($p = 0.005$). Analysis of variance showed the differences between controls and the various potassium concentrations to be statistically significant ($p = 0.05$). The results reported in Table XIV afford no evidence of cesium suppression increasing with potassium concentration, the differences between individual concentrations are not significant ($p = 0.05$).

At the risk of being repetitious, it is emphasized that the only difference between the larval rearing procedure followed in these experiments and the ^{137}Cs uptake measurements previously described, was the presence of potassium chloride in the rearing pans.

TABLE XIV

Effect of potassium concentration on uptake of ^{137}Cs by A. aegypti reared at 25°C. Larvae fed dog chow labelled with ^{137}Cs. Specific activity of food = 10^6CPM/g. Activity and wet weights reported as mean ± one standard deviation

1 Time-hrs	2 Instar	3 Mean wt. mg	4 Mean Activity CPM	5 Mean Activity/mg 4/3	6 Remarks
CONTROLS					
22	1st	-	3.2 ± 0.440	-	N = 6
48	1st instar exuvia	-	10 ± 6.84	-	N = 6
48	2nd	-	42 ± 14.8	-	N = 5
74	2nd	-	99 ± 11.0	-	N = 4
74	2nd instar exuvia	-	6.5 ± 1.50	-	N = 5
74	3rd	0.41 ± 0.08	137 ± 36.0	335	N = 6
96	3rd	0.55 ± 0.11	325 ± 72.5	590	N = 6
96	4th	0.99 ± 0.08	438 ± 64.5	442	N = 6
117	4th	1.21 ± 0.10	345 ± 21.5	286	N = 6
117	4th instar exuvia	-	12 ± 6.10	-	N = 5
117	♂ pupa	0.76 ± 0.22	172 ± 34.5	227	N = 6
117	♀ pupa	1.17 ± 0.11	285 ± 20.5	245	N = 5
168	♂ adult	0.60 ± 0.07	106 ± 22.0	177	N = 5
192	♀ adult	1.03 ± 0.09	181 ± 29.5	181	N = 5
0.001M					
22	1st	-	1.7 ± 0.791	-	N = 6
48	1st instar exuvia	-	22 ± 7.95	-	N = 6
48	2nd	-	43 ± 19.7	-	N = 6
74	2nd	-	56 ± 9.73	-	N = 6
74	2nd instar exuvia	-	11 ± 4.05	-	N = 6

TABLE XIV Cont'd.

1 Time-hrs	2 Instar	3 Mean wt. mg	4 Mean Activity CPM	5 Mean Activity/mg 4/3	6 Remarks
74	3rd	0.48 ± 0.05	59 ± 8.15	124	N = 6
96	3rd	0.73 ± 0.08	67 ± 8.57	92	N = 5
96	4th	1.15 ± 0.14	103 ± 11.7	90	N = 6
117	4th	1.17 ± 0.23	180 ± 28.4	154	N = 6
117	4th instar skin	-	43 ± 10.4	-	N = 6
117	♂ pupa	0.71 ± 0.03	63 ± 10.5	89	N = 6
117	♀ pupa	1.19 ± 0.08	119 ± 21.6	100	N = 6
168	♂ adult	0.55 ± 0.05	38 ± 18.7	69	N = 6
192	♀ adult	0.88 ± 0.13	71 ± 41.4	80	N = 6
0.0025M					
22	1st	-	2.4 ± 1.52	-	N = 6
48	1st instar skin	-	11 ± 5.34	-	N = 6
48	2nd	-	0.53 ± 0.179	-	N = 6
74	2nd	-	49 ± 5.66	-	N = 6
74	2nd instar skin	-	9.0 ± 3.92	-	N = 6
74	3rd	0.44 ± 0.06	65 ± 13.1	148	N = 6
97	3rd	0.69 ± 0.08	71 ± 13.8	102	N = 6
97	4th	0.97 ± 0.09	131 ± 18.0	135	N = 6
117	4th	1.27 ± 0.11	126 ± 21.8	99	N = 6
117	4th instar skin	-	20 ± 6.89	-	N = 6
117	♂ pupa	0.70 ± 0.08	56 ± 10.9	80	N = 6
144	♀ pupa	1.18 ± 0.15	111 ± 18.6	94	N = 5
168	♂ adult	0.57 ± 0.04	39 ± 9.78	68	N = 6
192	♀ adult	0.90 ± 0.03	39 ± 1.06	43	N = 2

TABLE XIV Cont'd.

1 Time-hrs	2 Instar	3 Mean wt. mg	4 Mean Activity CPM	5 Mean Activity/mg 4/3	6 Remarks
0.005M					
22	1st	-	1.0 ± 0.600	-	N = 6
48	1st instar exuvia	-	9.6 ± 2.34	-	N = 6
48	2nd	-	39 ± 10.4	-	N = 3
74	2nd	-	32 ± 9.04	-	N = 6
74	2nd instar exuvia	-	13 ± 4.22	-	N = 6
74	3rd	0.48 ± 0.07	61 ± 16.3	127	N = 6
97	3rd	0.66 ± 0.10	68 ± 5.48	104	N = 6
97	4th	1.15 ± 0.06	102 ± 10.4	89	N = 6
117	4th	1.31 ± 0.12	129 ± 13.5	98	N = 5
117	4th instar exuvia	-	18 ± 4.71	-	N = 5
117	♂ pupa	0.68 ± 0.08	56 ± 6.70	83	N = 6
117	♀ pupa	1.14 ± 0.11	90 ± 12.8	79	N = 6
168	♂ adult	0.55 ± 0.06	26 ± 9.31	47	N = 6
192	♀ adult	0.84 ± 0.11	41 ± 13.3	49	N = 4

The failure to detect a graded response to increasing potassium concentration, at least at the lower molarities, was unexpected. It was prudent, therefore, to determine the amount of potassium in the labeled dog-meal which had been fed to the larvae. Ground dog-meal is a difficult matrix in which to measure potassium. Flame photometric measurement is precluded because of the presence of many interfering ions. Ordinary atomic absorption spectrometry was precluded for similar reasons. A satisfactory X-ray fluorescence technique was developed by the Analytical Branch, W.N.R.E., and I am indebted to Messrs. B. Stewart and G. Smith for the potassium measures given in Table XV. When it was discovered that the food contained a significant amount of potassium, 0.4 mg/g dry weight, an experiment was designed to determine the amount of potassium and ^{137}Cs that leached out of the food into the rearing medium in 48 hrs, that is between feedings. A series of trays containing measured amounts of larvae food in 250 ml of distilled water, and in 0.01 and 0.05 M potassium chloride solutions, were set aside at 25°C. Samples of each supernatant were taken after 48 hrs, filtered, and their potassium content estimated by the X-ray fluorescent technique and their radiocesium content measured by total-beta counting. These data also are given in Table XV, where it will be noted that the potassium concentration of the external medium had apparently negligible effect on the amount of potassium which leached out of the food.

TABLE XV

Potassium and ^{137}Cs leached at 25°C from the food of Aedes aegypti larva

	Potassium*	^{137}Cs- Beta counts
Vacuumed dried food	0.4 mg/g	10^5 cpm/g
Supernatant - after food leached in distilled water for 48 hrs	0.2 mg/ml	10^4 cpm/ml
Supernatant - after food leached in 0.01M KCl solution for 48 hrs	0.7 mg/ml	10^4 cpm/ml
Supernatant - after food leached in 0.05M KCl solution for 48 hrs	3.3 mg/ml	10^4 cpm/ml

*measured by Analytical Branch, Research and Development Division, W.N.R.E., using an X-ray fluorescent technique

The larvae of A. aegypti, a freshwater mosquito species, may be reared in sea-water hypertonic to the normal blood (Wigglesworth, 1965), which produces an osmotic pressure equivalent to that of 1.5% sodium chloride. We may conclude, therefore, that the potassium concentrations used in these experiments, while possibly subjecting the larvae to a physiological stress, did not cause lethal osmotic pressures (Wigglesworth, 1938). Normal A. aegypti larvae shrink in hypertonic solutions, but those ligated at the rear or at both ends do not (Wigglesworth, 1933). This finding indicates that the integument of the larvae is impermeable to water. Furthermore, larvae which have had their anal papillae destroyed by exposure to strong salt solutions are also osmotically inert. These and other findings lead Wigglesworth to conclude that the anal papillae are the principle water-absorbing organs of the larvae.

The mechanism by which A. aegypti larvae absorb inorganic ions from freshwater into the haemolymph against a concentration gradient was investigated by Treherne (1954) using ^{24}Na. His work confirmed Wigglesworth's non-isotopic demonstration that the papillae were responsible for most inorganic ion uptake. Destruction of the papillae reduced accumulation by 80%. Blocking the mouthparts effected a further reduction of 15%, demonstrating that uptake from the gut was also significant (Hasset and Jenkins, 1951; Treherne, 1954; and Stobbart, 1959). The magnitude of the sodium flux is apparently dependent on feeding, being reduced sevenfold by starvation. Nevertheless, sodium balance was retained by the starved larvae (Stobbart,

1959, 1960), suggesting that both entry and exit of sodium are metabolically controlled. A twenty-five fold increase in external potassium level had no effect on the rate of ^{24}Na uptake, indicating the operation of a highly specific sodium-pump that is insensitive to potassium concentration (O'Brien and Wolfe, 1963). The experimental evidence which has been cited demonstrates an active transport mechanism for sodium ions, but not potassium. Presumably potassium is transported across the papilliary membrane by a diffusion process only. The ionic radius of cesium is 1.67 Å, that of potassium and sodium is 1.33 Å and 0.97 Å respectively (Weast, 1967). Thus the ionic radius of cesium is closer to that of potassium, and it is reasonable to propose in the absence of evidence to the contrary that cesium also crosses the papillary membrane by diffusion.

The suppression of ^{137}Cs uptake observed may be explained by postulating that diffusion of cesium across the papillary membrane is suppressed or 'blocked' by potassium ions at the concentrations employed in these experiments, that is an isotopic dilution effect. The amount of radiocesium which was accumulated resulted from intake via the gut. The data in Tables XII and XIII are reproduced in the histogram shown in Figure 12. In this figure, the maximum level of ^{137}Cs accumulated by larvae in the various potassium concentrations at one sampling-time and by male pupae, are shown as a fraction of the corresponding value obtained for the controls. The increased

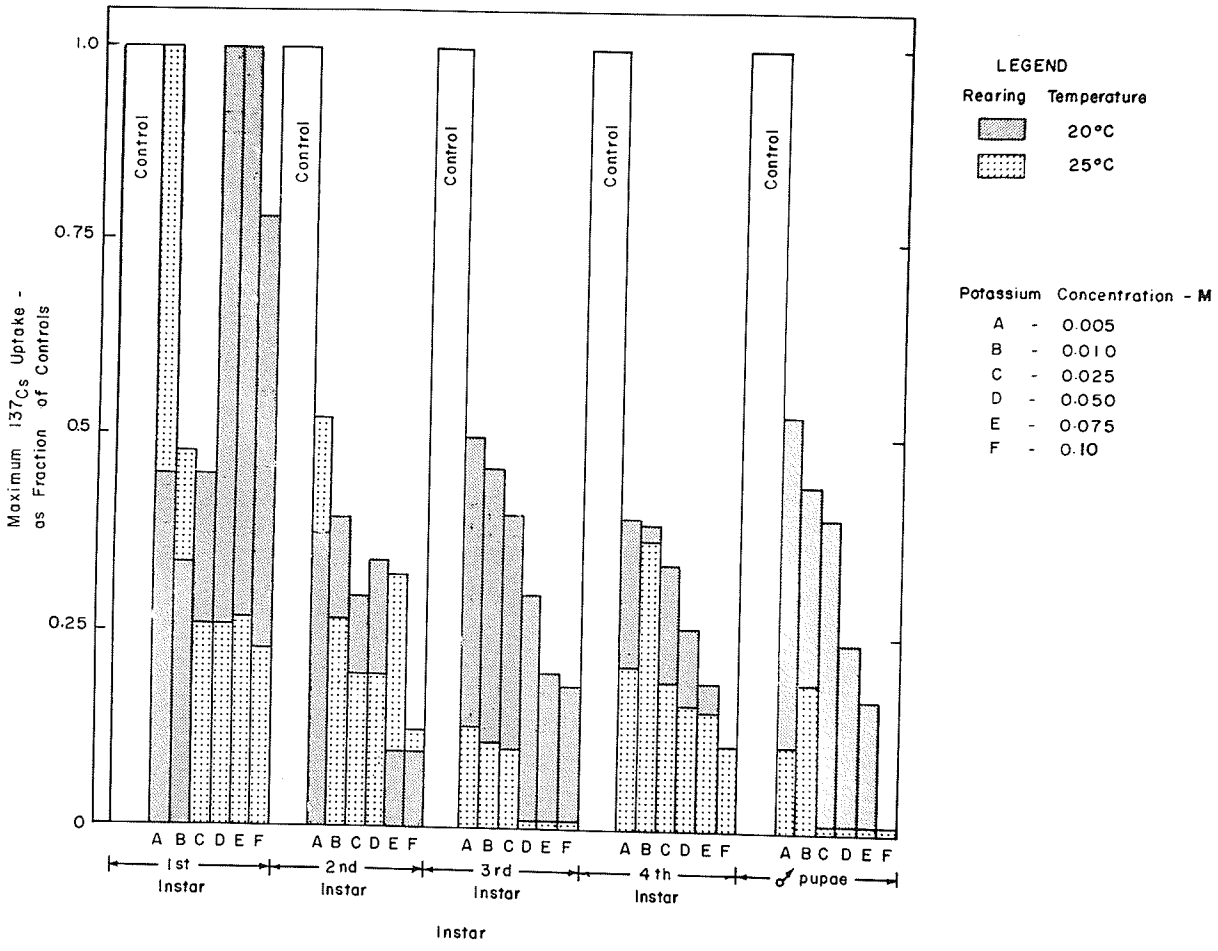

Figure 12

Histogram showing effect of potassium concentration (M/1) in rearing medium on uptake of ^{137}Cs by A. aegypti larvae reared at 20°C and 25°C. Uptake of larvae and pupae are expressed as a fraction of the controls. No measurements were obtained for 4th instar larvae reared at 20°C in 0.10 M potassium solution

suppression observed at 25°C for 3rd and 4th instar larvae and pupae, may be a reflection of increased ion mobility at the higher temperature. It will also be seen from Figure 12 that the amount of ^{137}Cs accumulated in the various potassium concentrations at 25°C averages about 15% of the control-level, comparable to the value obtained for ^{24}Na uptake via the gut. It is concluded that potassium is translocated across the anal papillae of A. aegypti larvae in preference to cesium. In a field situation, one could expect that larvae growing in waters having a low mono-valent ion content will accumulate more ^{137}Cs than larvae maturing in waters containing significant amounts of potassium or sodium.

Calculation of the amount of food consumed by A. aegypti larvae, using the specific activities given in column 5 of Table VII and the biological half-lives calculated from Figure 9, gave values about one order of magnitude lower than those derived from published work (Brust, 1968). This is explained by the finding that the majority of the larva's radiocesium uptake occurs across the papillary membrane, not via the gut. Consequently, application of the radiotracer method to estimation of larval food consumption requires knowledge of the fraction of nuclide accumulated via the gut. In these experiments, the larva's radiocesium body-burden resulted from accumulation of ^{137}Cs which leached into the rearing medium from the food, and not assimilation from ingested food. In a natural situation such as the radioactive pond, papilliary uptake should not be dominant, there being little ^{137}Cs in the water.

CHAPTER VII

FIELD STUDIES

INTRODUCTION

In conjunction with the laboratory studies which have been described, investigations of the levels of ^{137}Cs accumulated by the insect component of the radioactive pond community were pursued throughout the summer of 1967 and 1968. A number of radiocesium measurements were also made on the pond algae.

A general description of the radioactive and non-radioactive ponds has been given under Methods, particularly their construction, and the introduction of the contaminant into the radioactive pond. A more detailed account of the major abiotic factors pertinent to the ponds follows.

Abiotic Factors

Both ponds are situated in Section 28 of the W.N.R.E. Controlled Area. Appendix C gives the precipitation, and the air temperatures and humidity recorded at 1.5 m above ground level, measured during the summer months of 1967 and 1968 at the climatological station located about 50 m due south of the radioactive pond. The spring, summer and autumn surface and pond-bottom water termperatures for both ponds are shown in Figure 13. Adopting the practice suggested by Williams (1951, 1961), the temperatures plotted in this figure are the means of the preceeding five day period.

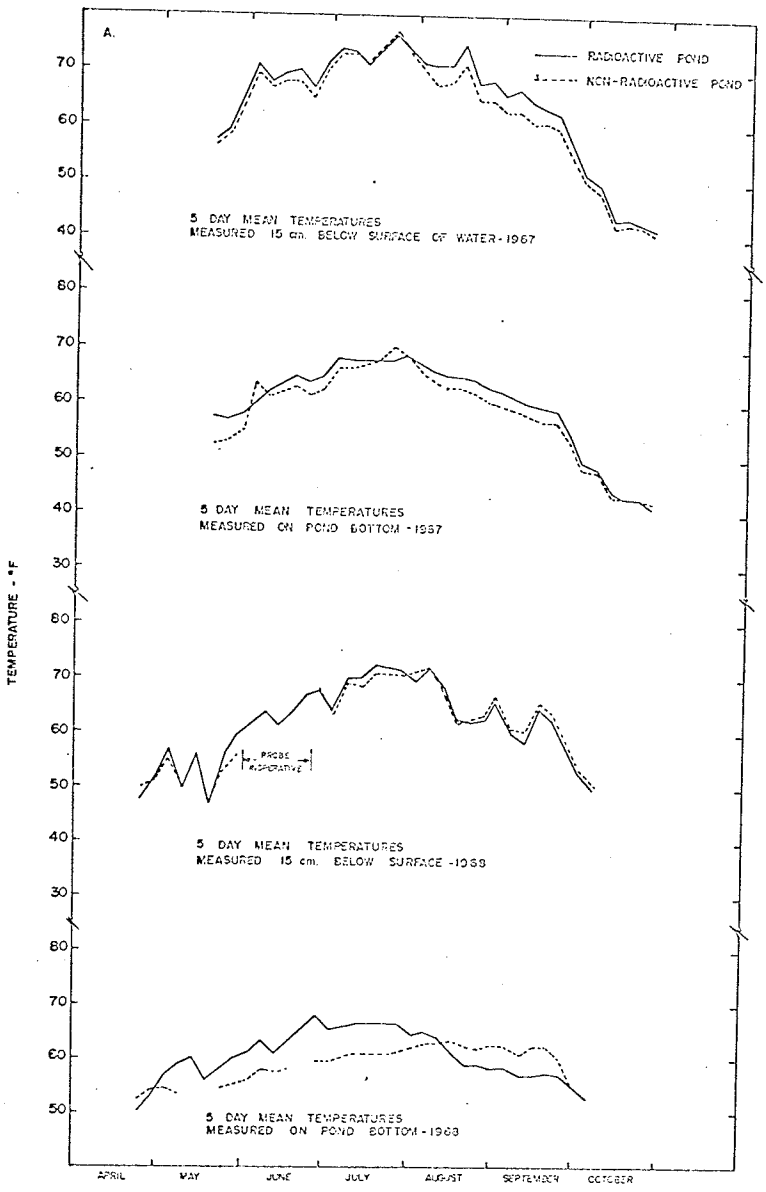

Figure 13

Surface and bottom temperatures of the radioactive and non-radioactive ponds recorded during the summer of 1967 and 1968.

Seven and ten day mean values have also been abstracted from these data with a view to future correlation of insect activity with weather conditions, in cooperation with the W.N.R.E. meteorology group.

The surface temperature probes were maintained at 15 cm below the water surface by means of a buoy; which also shaded from incident sunlight. The bottom temperature probes were anchored on the pond-bottom. The two probes in the radioactive pond were inter-calibrated with the two in the non-radioactive pond, and the calibration checked from time to time. Inter-probe agreement was better than $1^{o}F$. The five day mean temperatures of both ponds, measured 15 cm beneath the surface and on the pond bottom, were similar for the two ponds (Figure 13). However, the difference between the surface and bottom temperatures during the months of August and September are significant. This difference is attributed to the presence of algal growth (Westlake, 1966). During these months, nine-tenths of the non-radioactive pond surface was covered with a heavy bloom of algae, whereas that of the radioactive pond had but a limited growth at the pond margin. Thus the heavier algal growth in the non-radioactive pond probably reflected a significant fraction of incident solar radiation, and also shielded the bottom-temperature probe to a greater extent. The bottom-temperature of the non-radioactive pond peaked significantly above that of the radio-active pond during the period 30 May to 3 June 1967 (Figure 13). The ambient air temperature measured 0.5 m above ground-surface also exceeded by $10^{o}F$ that of the preceeding and suceeding five day

intervals (Reimer, 1968). At the same time, the water-depth of the non-radioactive pond was 18 cm less than that of the radioactive pond. Therefore, the bottom temperature of the former pond would warm up and cool, faster than that of the radioactive pond.

The depth of water in each pond was routinely measured. Water depths for 1967 and 1968 are summarized in Figure 14, where it will be seen that the depth-difference between the ponds was consistent. The total rainfall in the period May to September, was greater in 1968 than in 1967, 48.2 cm compared with 14.2 cm. Heavy rainfall in 1968 began in early June and is reflected in the sharp increase in pond-depths (Figure 14).

The dissolved oxygen (DO) content of the water in each pond was frequently measured in situ during the months of April to September, in 1967 and 1968. DO measurements were made with a Yellow Spring Instrument Company polarographic cell. The precision of the measurements was $\pm$ 6%. Instrument calibration was checked by comparative DO determinations made using the Winkler titration method described by Carpenter (1965). Most of the dissolved oxygen measurements were made on the non-radioactive pond, to avoid contaminating the DO instrument with radiocesium; the polarographic cell was difficult to decontaminate. However, enough DO measurements were made on both ponds at the same time to conclude that the dissolved oxygen levels measured at several depths in either pond differed by less than 10%. The maximum DO level measured was 12 mg/l at 10 cm beneath the surface. The lowest level was recorded in 1967, 7.0 mg/l

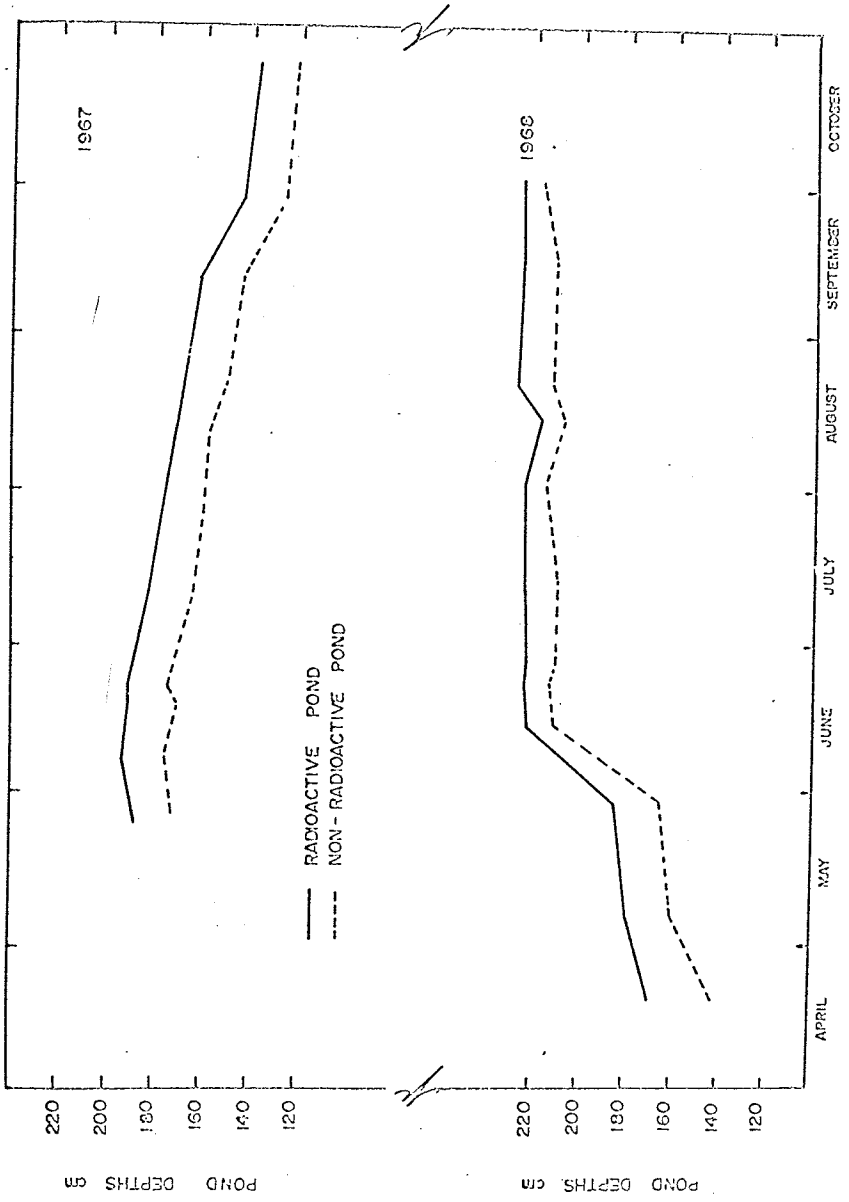

Figure 14

Water depths in cm. measured at the centre of the radioactive and non-radioactive ponds during the summer of 1967 and 1968.

at the bottom of the non-radioactive pond.

Comparative measurements of light intensity were made 15 cm below the surface and on the bottom of both ponds, using an instrument built at W.N.R.E., based on the 4π light meter design described by Maddux (1966). Light intensity measurements were taken when the sun was at its meridian, except when the sky was overcast. Fewer than five percent of the measurements made on the radioactive pond differed by more than the precision of the instrument from comcomitant readings taken in the non-radioactive pond. The average difference in light intensity between pond surface and bottom readings was 18 ± 5 foot candles.

Samples of water taken from both ponds were sent to the Analytical Branch, W.N.R.E., for analyses of non-radioactive, i.e., stable, anions and cations. In spite of the heavy rains in 1968, their concentration varied but little from measurements made in 1967. These data are summarized in Table XVI.

Flora in the Vicinity of the Ponds

As described previously, both ponds were dug in an old farm-field in the early stages of succession to mixed decideous forest. The field was in its second or third year of an undisturbed weedy condition. The average height of the trees in the immediate vicinity of both ponds was 3.5 to 4.0 m, diameter at breast height 2-3 cm. When the ponds were dug, the ground was cleared to a distance of 5 m beyond the pond perimeters. Twelve sampling stakes were placed 10 m apart, around the outer edge of this cleared strip that

TABLE XVI

Summary of anion and cation concentrations in water samples taken from the radioactive and non-radioactive ponds, summer 1967 and 1968. Precision of analysis ± 5%

ANION - mg/l			CATION - mg/l		
Chloride	-	3.5	Calcium	-	51.6
Nitrate	-	3.3	Cobalt	-	0.06
Phosphate	-	1.5	Chromium	-	<0.02
Sulphate	-	750.0	Cesium	-	<0.01
			Copper	-	<0.02
			Iron	-	<0.02
			Magnesium	-	71.9
			Manganese	-	0.01
			Nickel	-	<0.02
			Potassium	-	13.7
			Sodium	-	30.0
			Strontium	-	0.7
			Zinc	-	0.44

surrounded each pond. Sampling was done, therefore, within 10 m wide strip surrounding each pond. Emergent and sub-mergent vegetation was not included in the survey. Species described as dominant (D) comprised at least 15% of the vegetation within a 5 m radius of each sampling stake.

The only vegetation which grew in the margin of the radioactive pond was cattail (Typha sp.). This species was not growing in the non-radioactive pond in 1967 or 1968. Vegetation encompassed the ponds on the west, north and east sides only. The south side of both ponds bordered on the open field. The vegetation surrounding the radioactive pond may be considered representative of both ponds, and the immediate area (Dugle, 1968). Trees were present, but not dominant within the vicinity of the pond perimeters. They offered little canopy cover, and none overhung either of the ponds. Shrubs too, were present but none were dominant with the one exception of two Salix spp., at one sampling stake. The dominant herbs were Sonchus arvensis, the wild strawberry Fragaria viginerea, Solidago giganteus, and Achillea lanulosa. The species growing about the radioactive pond are listed in Appendix 'F'. Specimens were deposited in the W.N.R.E. herbarium.

Algae in the Ponds

The surface and bottom algal growths in both ponds were sampled in 1967 and 1968. In 1967, the dominant alga in both ponds was an Oedogonium sp., associated with Cladophora (Walker, 1968). A few epiphytes and desmids were also present. More extensive sampling

was carried out in 1968 and the identifications are listed in Appendix 'G'. The Arthropoda and Protozoa associated with these samples are listed in Appendix 'H'.

A number of algae were found only in the radioactive pond:

> Closteriopis sp., Gleoocystis sp., and two species of Desmids, Class CHLOROPHYCEAE
>
> Hydrosera sp., Class BACILLARIOPHYCEAE
> A red colored colonial, and a few ephiphytic
>
> Oscillatoria sp., Class MYXOPHYCEAE, and one Euglenia sp. (Lepocinclis actuta Presc.), Class EUGLENACEAE, were also found in the radioactive pond samples only.

Of potentially greater interest are the species abundant in the non-radioactive pond, but not found in the samples taken from the radioactive pond:

> Scendemus, two species of Cladophora provisionally identified as #3 and #6, Haematococcus lacustris (Girod.), and an unidentified filamentous form, all of the Class CHLOROPHYCEAE.
>
> Fragilaria, and Calonesis, Class BACILLARIOPHYCEAE, Phaeothamnion sp., Class CHRYSOPHYCEAE.
>
> Chamaesiphon ?incrustans Grunow, Gloeocapsa ?rupestris Kuetzing, and a Oscillatoria sp., Class MYXOPHYCEAE.
>
> An unidentified species of Euglena, Class EUGLENOPHYCEAE.

The radiation field in the radioactive pond might be responsible for the different species observed between the two ponds.

A difference in the occurrence of small (microscopic) animals in the two ponds was also noted. TARDIGRADA were abundant in all the algae samples taken from the non-radioactive pond. None were found in the samples obtained from the radioactive pond. Similarly, the Protozoans Stentor-polymorphus and a species of Lacrymaria, were found in non-radioactive pond algal samples, but not in those taken from the radioactive pond.

Pond Insect Fauna

The major aquatic insect types samples from both ponds, but excluding those species occupying habitats in the pond-margin, are listed in Appendix 'I'. No species differences were observed between ponds. Mosquito larvae were absent from both ponds in 1967 and 1968. Species identification concentrated on the Hemiptera and the larger beetles. Except where stated otherwise, taxonomic keys used to identify specimens were those of Pennak (1953), Ward and Whipple (1959), Needham and Needham (1962), and Brooks and Kelton (1967) for the Heteroptera of Manitoba. Specimens were preserved for future positive identification, and comparison.

Other Fauna

Tadpoles were plentiful in the ponds in 1967, and in the non-radioactive pond in 1968. The most abundant species was Bufo ? americanus, the common toad, and those of the green frog. Frogs and

toads were obtainable from both ponds in 1967 and 1968. By freeze-up, in 1968, no fish were present in either pond. Franklin ground squirrels (Spermophilus franklinii), and two species of field mice, Clethriomonys gapperi and Zapus hudsonius, were live-trapped in the immediate vicinity of the radioactive pond to determine if this pond was a source of drinking water for small mammals (Iverson, 1968). The tracks of coyotes, deer and bear were seen around the margins of both ponds.

CHAPTER VIII

THE FATE OF ^{137}Cs ADDED IN THE RADIOACTIVE POND

Distribution in the Water

The radioactive pond was contaminated on 11 May, 1967, by mixing 0.5 Ci of ^{137}Cs into the water. Replicate samples of pond water were taken two hours later and analysed for radiocesium by gamma spectroscopy (see Methods). The average concentration of radiocesium achieved was 6.4×10^{-3} μCi/ml, or about 32 times the occupational 168 hr maximum permissible concentration in water recommended by the International Commission (ICRP, 1959). Prior to addition of the radionuclide, radiochemical analyses of samples of water from both ponds showed the concentrations of long-lived biologically important nuclides to be:

Total-beta activity (as ^{40}K equivalent) - 4.3×10^{-8} μCi/ml

^{137}Cs - 2×10^{-10} μCi/ml

^{90}Sr - 1×10^{-10} μCi/ml

Thus, the only source of radioactive contamination, prior to the addition of radiocesium to one of the ponds, was nuclear fallout.

Following the addition of ^{137}Cs to the radioactive pond, the water was sampled to establish the rate at which the concentration of radiocesium decreased. These measurements are summarized in Figure 15, where the ^{137}Cs activity of the water in disintegrations per minute (dpm) per ml is plotted against time. Only the measurements made on samples taken in 1967 are shown in this graph. By

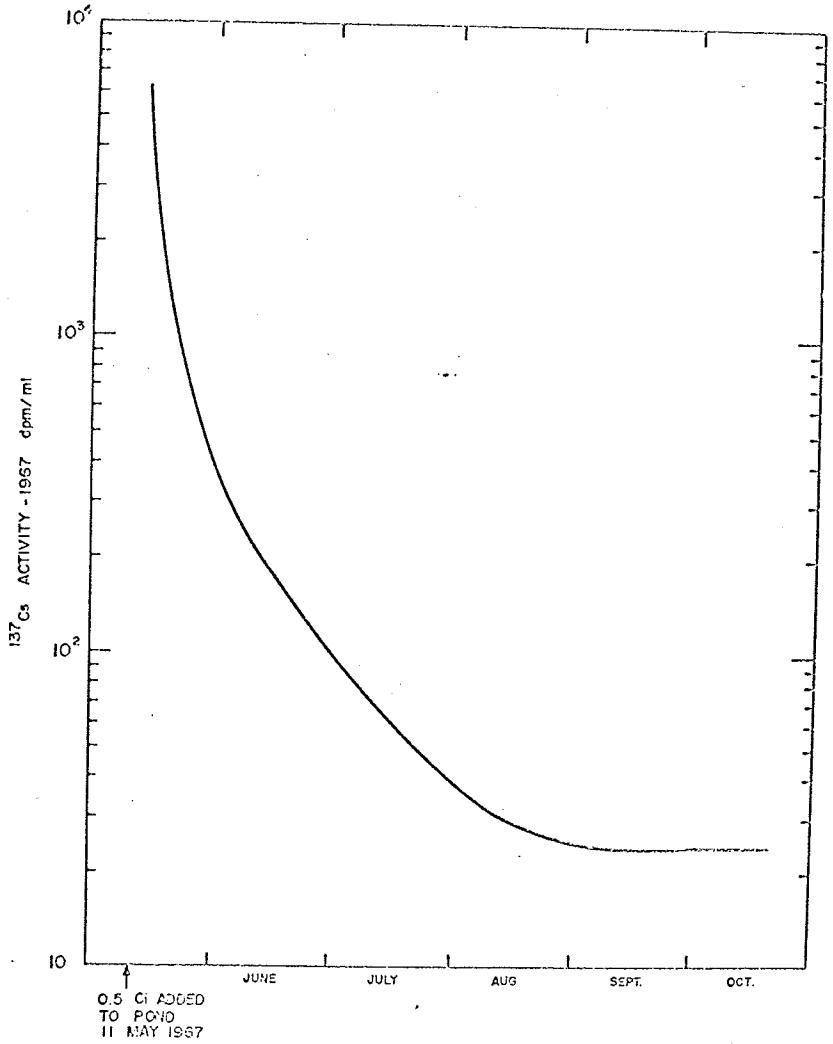

Figure 15

Decrease of ^{137}Cs concentration in the radioactive pond water after the addition of 0.5 Ci ^{137}Cs on 11 May 1967.

April, 1968, the decrease in ^{137}Cs concentration had stabilized at 3 $\pm$ 1.5 dpm/ml, the limit of sensitivity of the method of measurement. The value, throughout 1968, is only one order of magnitude higher than the level measured in non-radioactive pond water, determined using a gravimetric method of radiochemical analysis (Guthrie and Grummit, 1963).

Analysis of the curve in Figure 15, using graphical methods (ORE, 1963) showed the radiocesium concentration in radioactive pond water decreased at two different rates. The major decrease occurred during the 20 day period succeeding addition of the nuclide to the pond, and compares very well with rates described by several workers investigating the uptake of ^{137}Cs and other radionuclides by soils and river sediments (Clanton and Reynolds, 1963; Tamura, 1964; Lominick and Gardiner, 1965; Lomenick and Tamura, 1965, and Pickering et al., 1966). It is concluded from Figure 15, therefore, that the initial decrease in ^{137}Cs in the pond water was the result of sorption on to pond sediment. The disappearance of ^{137}Cs from the water continued at a slower rate from day 30 to about day 75, and then apparently reached steady-state. The latter decrease is attributed to uptake and loss of radiocesium by the pond biota (Davis, 1963; Seymour, 1964).

Distribution of ^{137}Cs in the Pond-bottom Sediments

The pond-bottom sediment was sampled once a month during the months May to September in 1967 and 1968, at ten randomly selected

locations (see Methods). The mean specific total-beta activity of mud samples taken from the non-radioactive pond in 1967 was 9.6 cpm/g, and 9.0 cpm/g in 1968. These values are not significantly different at the 95% confidence level. The specific activities of the mud samples taken from the radioactive pond are summarized in Appendix 'J'. These measurements confirm the postulate that most of the ^{137}Cs added to the radioactive pond was sorbed by the pond-mud. It will also be noted from the results recorded in Appendix (J) that radiocesium sorption by radioactive pond mud was non-uniform. This finding will be discussed further under distribution of radiation dose in the radioactive pond (Chapter IX).

Uptake of ^{137}Cs by Algae

Algal samples were taken from both experimental ponds, and from other locations in the W.N.R.E. Controlled Area, in May 1967, and in July, 1967 and 1968. It was not possible at the time of analysis to identify the species contained in the samples, but it was established that Cladophora was the dominant one present (Dugle, 1968). The samples were dried in a hot-air oven overnight at 105°C, compressed into pellets and the ^{137}Cs activity determined by gamma spectrometry (Guthrie and Grummitt, 1963). The results are summarized in Table XVII.

There was no significant change in the level of radiocesium in the algal samples obtained from the non-radioactive pond in 1967 and 1968, and it is considered that radioactive fallout was the major source of the nuclide. Algae samples obtained from the gravel pit in Section 27 of the W.N.R.E. Controlled Area contained only

TABLE XVII

^{137}Cs activity of Algae spp. taken from the experimental ponds and other localities within the W.N.R.E. Controlled Area. Activity measured by gamma spectroscopy, using oven dried samples

Month sampled	Location	Wet wt g	Activity dpm/g
1967			
May	Radioactive pond	3.85	7.3×10^6 dry wt
May	Non-radioactive pond	14.7	6.29×10^2 dry wt
May	Pond-Controlled Area	-	4.64×10^2 dry wt
July	Radioactive pond	34.5	5.80×10^5 dry wt
July	Non-radioactive pond	15.3	2.62×10^2 dry wt
July	Winnipeg River, near W.N.R.E.	10.4	50.6 dry wt
July	Gravel Pit, Sect. no. 27	16.1	1.28×10^2 dry wt
1968			
July	Radioactive pond	-	5.13×10^4 dry wt
July	Non-radioactive pond	-	2.10×10^2 dry wt

half as much ^{137}Cs as did the non-radioactive pond samples (Table XVII). This gravel pit, which is about 1 km. due east of the non-radioactive pond, is located in a discharge zone. Consequently, the residence time of water in this pond can be expected to be much lower than that of the non-radioactive pond. Neither would the gravel in the pit retain the nuclide for the same length of time. It will also be seen from Table XVII that the amount of radiocesium in algae taken from the Winnipeg River was much lower than the pond or gravel pit samples. This is a reflection of the lower concentration of ^{137}Cs in river water, compared to pond water; the amount of ^{137}Cs in fallout was greatest in April and May (Acres, 1968).

After the addition of ^{137}Cs to the radioactive pond, the level of the nuclide in the algae sampled from this pond increased four orders of magnitude, reflecting the rapid uptake of radiocesium by planktonic growth (Pendleton and Hanson, 1958). Two months later, the concentration of ^{137}Cs had decreased by one order of magnitude (Table XVII). By July 1968, the activity of the algal samples had decreased by another order of magnitude to about 5×10^4 dpm/g dry weight. When one compares the relative ^{137}Cs activities of pond water, pond mud, and algae, it is evident that a small, but significant, fraction of the radiocesium added to the radioactive pond was bound up in the algae. No precise data on the biomass of algae growth was obtained, nevertheless a first

approximation based on the weight of growth per square meter of surface suggests that less than 2% of the radiocesium added to the radioactive pond was bound up in algal growth.

Uptake of ^{137}Cs by Aquatic Insects

The uptake of radiocesium by the major insect species inhabiting the radioactive pond was followed in 1967 and 1968. The average amounts of the nuclide measured in more than 200 replicate samples are summarized in Table XVIII. The level of radiocesium in comparable species taken from the non-radioactive pond are not included in this table as they consistently showed no more than the natural background level, <0.1 cpm/insect. The amount of stable (non-radioactive) cesium measured in the insects was between 5 and 9 ug/insect. The most abundant large animal inhabiting the experimental ponds in 1967 and 1968 were frogs (Rana sp. and Bufo ? americanus). The mean total-beta activity of replicate samples of tadpoles taken from the non-radioactive pond during the summers of 1967 and 1968 was 0.8 cpm/tadpole. Table XIX gives the mean activities of juvenile and adult frog samples from the radioactive pond.

RESULTS AND DISCUSSION

Examination of Table XVIII shows that the activity of Chironomidae larvae sampled from the radioactive pond varied between 2.5 cpm and 786 cpm/larvae. Comparison of the locations from which

TABLE XVIII

^{137}Cs levels measured in aquatic insects sampled from the Radioactive Pond. Weights and activities reported as means ± standard error

Month sampled	Insect	Mean wt mg	Activity CPM	Specific activity CPM/g
1967				
May	Ceratopogonidae larvae	0.4 ± 0.05	0.3 ± 0.05	750
May	"	0.1 ± 0.05	<0.1	-
June	"	0.1 ± 0.05	0.1 ± 0.02	1×10^3
July	"	0.2 ± 0.05	0.1 ± 0.02	500
July	"	0.2 ± 0.02	0.1 ± 0.03	500
October	"	0.2 ± 0.02	0.2 ± 0.03	1×10^3
October	"	0.3 ± 0.05	0.1 ± 0.004	333
May	Chironomidae larvae (early instar)	0.27 ± 0.01	2.5 ± 0.7	9.3×10^3
May	"	0.80 ± 0.05	0.7 ± 0.1	875
June	"	0.10 ± 0.02	3.3 ± 0.9	3.3×10^4
June	"	2.5 ± 0.6	38 ± 11	1.5×10^4
September	"	0.51 ± 0.21	1.0 ± 0.6	2×10^3
September	"	1.7 ± 0.2	0.1 ± 0.05	59
October	"	0.48 ± 0.05	5.7 ± 1.2	1×10^4
October	"	0.21 ± 0.01	0.1 ± 0.01	4×10^3
1968				
May	"	5.5 ± 1.0	8.3 ± 2.2	1.5×10^3
May	"	0.31 ± 0.06	0.3 ± 0.1	968
July	"	1.6 ± 0.25	3.1 ± 0.8	1.9×10^3
August	"	2.7 ± 0.48	22 ± 8	8×10^3
October	"	2.7 ± 0.46	18 ± 9	6.7×10^3
1967				
May	Chironomidae larvae (late instar)	9.3 ± 0.88	786 ± 138	8.5×10^4
June	"	2.8 ± 0.58	192 ± 65	6.9×10^4

TABLE XVIII Cont'd.

Month sampled	Insect	Mean wt mg	Activity CPM	Specific activity CPM/g
1968				
June	Chironomidae larvae (late instar)	5.5 ± 0.54	76 ± 18	1.4×10^4
July	"	11.0 ± 1.3	29 ± 9	2.6×10^3
August	"	10.0 ± 1.4	223 ± 74	2.2×10^4
October	"	8.7 ± 0.97	4.5 ± 1.3	517
October	"	15.7 ± 2.0	201 ± 55	1.3×10^3
1967				
May	Chironomidae pupae	1.1 ± 0.12	7.9 ± 0.97	7.2×10^3
1968				
July	"	5.2 ± 0.18	1.8 ± 0.21	346
August	"	2.3 ± 0.24	0.9 ± 0.4	391
1967				
September	Odonata nymphs	41 ± 15	30 ± 9	732
October	"	43 ± 20	84 ± 20	2×10^3
1968				
May	"	25 ± 4	18 ± 7	720
June	"	132 ± 43	43 ± 26	326
July	"	58 ± 11	76 ± 18	1.3×10^3
July	"	96 ± 3	53 ± 9	552
August	"	279 ± 36	71 ± 12	254
August	"	61.5 ± 10	202 ± 50	3.3×10^3
October	"	650 ± 65	764 ± 76	1.2×10^3
July	Damselfly nymphs	13.6 ± 1.9	10.2 ± 2.0	750
1967				
June	Dytiscus larvae	23.6 ± 2.2	131 ± 34	5.6×10^3
September	"	1.5 ± 0.05	8.9 ± 1.1	5.9×10^3
October	"	1.5 ± 0.07	4.4 ± 0.4	2.9×10^3
1968				
June	"	308 ± 120	120 ± 45	390
July	"	25 ± 8	24 ± 2.3	960

TABLE XVIII Cont'd.

Month sampled	Insect	Mean wt mg	Activity CPM	Specific Activity CPM/g
1967				
May	Ephemeroptera nymphs	0.19 ± 0.03	152 ± 71	8×10^5
June	"	2.9 ± 0.5	66 ± 23	2.3×10^4
September	"	1.5 ± 0.08	14 ± 4	9.3×10^3
October	"	0.2 ± 0.05	3.1 ± 1	1.6×10^4
1968				
May	"	0.44 ± 0.14	8.2 ± 4	1.9×10^4
1967				
July	L. americanus 4th instar nymphs	865 ± 33	136 ± 16	157
July	L. americanus 1st instar exuvia	16 ± 4	19 ± 6	1.2×10^3
1967				
May	Gyrinidae adults	1.5 ± 0.08	7.7 ± 1.3	5.1×10^3
June	"	12.4 ± 1.0	5.9 ± 0.5	476
1968				
April	"	9.7 ± 0.6	10 ± 3	1×10^3
1967				
August	Dytiscus adults	3.4 ± 0.5 g	5390 ± 75	1.6×10^3
1968				
April	"	86.8 ± 0.2	109 ± 35	1.3×10^3
August	"	216 ± 38	126 ± 57	583
1967				
September	Corixidae adults	8.4 ± 1.2	0.48 ± 0.05	57
1968				
April	"	8.2 ± 3.0	31 ± 10	3.8×10^3
1967				
June	Gerridae adults	31.5 ± 1.9	23 ± 2	730
August	"	10.5 ± 1.8	6.5 ± 4	619
September	"	6.9 ± 1.6	1.9 ± 0.3	275

TABLE XVIII Cont'd.

Month sampled	Insect	Mean wt mg	Activity CPM	Specific Activity CPM/g
1968				
July	Gerridae adults	3.7 ± 1.0	1.8 ± 0.3	486
1967				
June	Notonectidae adults	5.1 ± 0.05	9.9 ± 1.1	1.9×10^3
July	"	38.6 ± 5.3	29 ± 5	751
July	"	172 ± 21	32 ± 6	186
August	"	70 ± 2	15 ± 1.0	214
September	"	89 ± 8	11 ± 3	124
October	"	81 ± 23	40 ± 14	494
1968				
June	"	85 ± 13	50 ± 2	588
July	"	19.6 ± 2	6.5 ± 0.7	332
August	"	32 ± 5	3.6 ± 0.4	113
1967				
June	L. americanus adults	5.6 ± 0.5 g	3219 ± 112	575
July	"	2.95 ± 0.07 g	1325 ± 68	449
August	"	3.83 ± 0.1 g	3598 ± 85	939

TABLE XIX

^{137}Cs levels measured in tadpoles and frogs sampled from the radioactive pond. Weights and activities reported as means ± standard error

Month sampled	Species	Mean weight mg	Activity CPM	Specific activity CPM/g
1967				
June	Tadpoles (*Bufo ? americanus*)	61 ± 6	11,806 ± 1557	1.9×10^5
July	"	85 ± 5	5318 ± 550	6.3×10^4
August	"	236 ± 16	6226 ± 756	2.6×10^4
September	"	160 ± 13	1611 ± 218	1×10^4
October	"	57 ± 4	1199 ± 165	2.1×10^4
1968				
June	"	38 ± 11	27 ± 8	711
1967				
October	Large Tadpoles (*Rana* sp.)	2.37 ± 0.17 g	9584 ± 200	4×10^3
1968				
June	"	0.74 ± 0.11 g	2654 ± 273	3.6×10^3
August	"	0.64 ± 0.01 g	978 ± 159	1.5×10^3
1967				
June	Small green frog (*Rana* sp.)	13.61 g (length-14.6cm)	23,170	1.7×10^3
June	Green frog (*Rana* sp.)	79.0 g (length-25cm)	4499	57

these samples were taken with the specific activity of the pond mud from the same location (Appendix 'J'), showed them to be highly correlated, r = 0.9850. Thus the level of ^{137}Cs in the larvae reflects the concentration of nuclide in their immediate environment. Chironomidae larvae, therefore, might serve as an indicator organism to measure radiocesium accumulation in the immediate vicinity of the larval environment. This finding is potentially useful to environmental radiation surveillance of aquatic habitats, as Chironomidae are an important link in the foodchain of fish (Comstock, 1940). Attempts to obtain representative samples of emerging Chironomidae adults for radiochemical analysis were unsuccessful.

Environmental radiation studies have demonstrated the tendency for radionuclides to accumulate rapidly in the first or primary producer, trophic level, and an apparent concentration of some of these radionuclides may occur in successive trophic levels (Schultz and Klement, 1963; Aberg and Hungate, 1966). Pendleton and co-workers in particular, have postulated that radiocesium is concentrated threefold at each successive level of foodchains, as it passes from primary producers to primary and secondary consumers and top carnivores (Pendleton, 1957, 1960, 1962; Pendleton and Hanson, 1958). This view has been challenged by other investigators. The work of Nelson and his colleagues (Nelson et al., 1967) suggests that trophic-level increases do "not appear to be a general rule" in aquatic environments, the observed differences being the result of

different foodchains in different habitats. These workers concluded that available data did not warrant general application of trophic-level increases to cesium in foodchains. A proposed food web linking the radioactive pond insect specimens listed in Table XVIII, is depicted in Figure 16. The insect families were assigned to trophic-levels according to feeding habits reported by Hungerford (1919), Galtsoff et al. (1937), Comstock (1940), and Brooks and Kelton (1967), and personal observations. Trophic-level is shown on the left side of the figure. The grand mean specific activity, in cpm/g, was calculated for each trophic-level by summing the specific activities of each insect family in that level. The grand means appear on the right side of Figure 16, and were calculated using the 1967 measurements from Table XVIII.

The grand mean of specific activity shows that each insect family at a given trophic-level can be grouped or classified within the same order of magnitude while the use of activity/insect does not permit such simple grouping. In general then, Figure 16 shows that insect families with specific activities in the same order of magnitude, were primarily feeding from the same trophic level of the food web in the radioactive pond. It is not inferred that the use of specific activity only enables one to assign an insect, or other aquatic organism, to the correct trophic level. However this tracer technique does provide a useful field method, in conjunction with the knowledge of natural feeding habits, for assigning organisms to the correct level of the predator part of a foodchain. In particular,

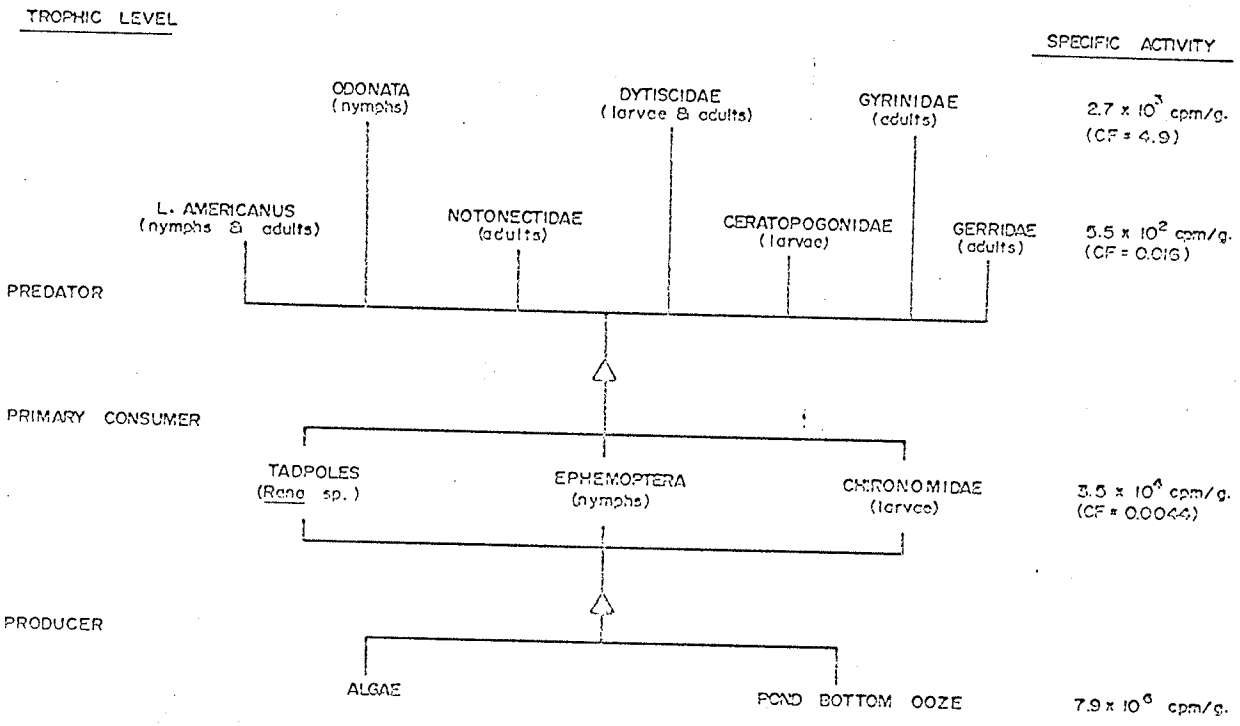

Figure 16

Statification, based on specific activity, and food-links for insects collected from the radioactive pond. CF = concentration factor.

this work shows that the tracer technique is a valuable method for confirming if two species assigned to the same trophic-level on the basis of known feeding habits, do indeed feed off the same level. Gyrinidae and Gerridae are surface-feeders (Galtsoff et al., 1937; Comstock, 1940), and it would be expected on the basis of this information that they feed from the same trophic level. However, the specific activity of the Gyrinidae specimens taken from the radioactive pond was about one order of magnitude greater than that of the Gerridae, therefore these insects must have been taking prey from different trophic levels. Similarly, field observation (Hungerford, 1919, for example), shows L. americanus to be the top carnivore among aquatic insect predators, but the species' specific activity (450 cpm/g) shows that it fed primarily at a lower level of the pond food web than the Dytiscidae (specific activity 2.7×10^3 cpm/g). It is noteworthy that the adults and juveniles, of both species, had the same specific activities. Nevertheless, although two species on the basis of specific activity appear to belong to different trophic-levels, it should not be hastily concluded they feed on different prey. Methods of food ingestion may account for the observed difference. L. americanus and the Dytiscidae are a case in point. No data on the fraction of ingested food assimilated by Dytiscidae have been reported in the literature. The studies of ^{137}Cs uptake by L. americanus which have been described (Chapter IV), showed that the fraction of the prey assimilated by this species is

quite small, compared to that of a terrestrial beetle, Chrysomela knabi Brown. Bourdeau et al. (1965) have studied the movements of radionuclides in irrigated ecosystems. They reported higher ^{137}Cs levels in Dytiscus marginalis Scop. than in the Hemipteran Notonecta glauca L., presumably the result of the smaller fraction of food assimilated by the latter species. Tadpoles were plentiful in the radioactive pond in 1967, and it is probable that L. americanus was feeding primarily on this prey. It is plausible, therefore, that the insect's low specific activity compared to that of the tadpoles (Figure 16) is a reflection of the small fraction of captured prey actually assimilated. It is concluded that specific activity measurements are a useful adjunct to feeding behavior information obtained by observation in the field, but they do not negate the requirement for thorough field observation.

The distribution of radiocesium along the food web depicted in Figure 16 can be conveniently described in terms of concentration factors, that is, the grand mean specific activity for any given trophic-level divided by the specific activity for the preceeding level:

Trophic Level	Specific Activity cpm/g	Concentration-Factor
Producer	7.9×10^6	-
1st Consumer (Herbivore)	3.5×10^4	0.0044
2nd Consumer (Predator)	5.5×10^2	0.016
3rd Consumer (Predator)	2.7×10^3	4.9

Increasing concentration factors imply that differences in levels of ^{137}Cs will be reduced at each transfer until, eventually, that of the top insect predator equals or exceeds the herbivore's-concentration. This situation was never achieved by the insects sampled from the radioactive pond. The concentration factors continued to increase through all trophic-levels. As suggested by Crossley and Shanks (1967), the minimal concentration of radiocesium is not found at the terminus of the simplified radioactive pond food web shown in Figure 16, but at an intermediate level.

Table XVIII also shows that with the exception of Dytiscidae larvae, the specific activities of none of the radioactive pond insects decreased appreciably in 1968. Neither did the levels of radiocesium in the tadpoles living in the radioactive pond, particularly Rana sp. (Table XXIX). The constancy of the specific activity over the winter months reflects low metabolic activity and concommitant reduced rate of radiocesium elimination. It also indicates that a reservoir of exchangeable ^{137}Cs must be available in the radioactive pond. If not, the specific activity of the insects should have dropped significantly by early summer of 1968. The reason why the specific activity of Dytiscidae larvae was significantly less in 1968 ($p = 0.05$) compared with the previous year, is not apparent.

The mean specific activity of Bufo ? americanus tadpoles (Table XXIX) was 6.2×10^4 dpm/g, a value which compares favorably with that of other primary consumers shown in Figure 16. The

specific activity of the large Rana sp. tadpoles in 1967 was 3.8×10^3 cpm/g, which suggests this species' primary food source was not the same as the toad tadpoles; perhaps it was the pond-bottom ooze.

Transfer of ^{137}Cs from the Radioactive Pond to Land

Peredel'skii and Bogatyrev (1959a, b) have postulated that a significant amount of radiocesium and other nuclides could enter terrestrial foodchains when adult aquatic insects emerge from ponds contaminated with radioactivity. Chironomidae were the most numerous insect species in the radioactive pond. Attempts to measure the potential contribution of these insects to terrestrial foodchains were unsuccessful. The adults all emerged within a few days, and because of their small mass, the activity of the individual insects was too small to give reliable counting statistics. Consequently, it appeared that a larger and heavier insect would be necessary to demonstrate removal of radioactivity from the pond via emerged adults. Tabanidae are numerous within the W.N.R.E. Controlled Area, particularly in the vicinity of the experimental ponds. The juvenile stages of this family are aquatic (Comstock, 1940), and the average dry weight of the adults was about 60 mg. A trap (Thorsteinson et al., 1965) supplied by Dr. A.J. Thorsteinson, Department of Entomology, University of Manitoba, was set up near the perimeter of the radioactive pond to catch newly emerged Tabanidae. Replicate samples of 10 horseflies were randomly selected from each

day's catch, throughout June and July, and the first week of August 1968. The samples were dried overnight in a calcium chloride dessicator, weighed, and their radioactivity assayed by total-beta counting (see Methods). Horseflies were also randomly collected in the town of Pinawa, about 10 miles east of the radioactive pond, and served as controls. The predominant species in the horsefly samples were Hybomitra epistates (Osten Sacken) and H. affinis (Kirby). The horsefly samples were not sorted by species before analysis.

The total-beta activities of the samples are summarized in Table XX. Those samples whose mean is significantly different from the controls, $p = 0.05$ and $p = 0.01$, are marked 'X' in this table. By Student's 't'-test, 14 of the 26 replicate samples obtained at the radioactive pond were significantly different from Tabanidae collected at Pinawa, $p = 0.05$. Eleven of these differed significantly from the controls at $p = 0.01$. It is also apparent from Table XX that almost all the samples whose mean values differed from that of the controls were caught in July. Clearly a significant fraction of Tabanidae caught in the trap emerged from the radioactive pond, and did so during the month of July. The samples were not analyzed by species, to determine if different amounts of ^{137}Cs accumulated were different. Live-traps to capture small mammals were also set around the perimeter of the radioactive pond throughout the spring and summer of 1968. All the animals captured contained only

TABLE XX

Total-beta activity measured in Tabanidae trapped beside the radioactive pond and at Pinawa, Manitoba. Weights and activities reported as means ± standard error. X indicates significant difference from controls

Month sampled	Location	Mean weight mg	Activity CPM	Different from controls	
				p=0.05	p=0.01
June and July 1968	Controls, taken at Pinawa, Man.	33.4 ± 2.1	1.12 ± 0.17	-	-
1968					
17 June	Radioactive pond	80.8 ± 6.8	8.4 ± 1.6	X	X
18 June	"	24.5 ± 1.5	0.83 ± 0.16	-	-
20 June	"	38.5 ± 3.8	1.0 ± 0.14	-	-
24 June	"	36.2 ± 4.2	5.8 ± 4.0	-	-
25 June	"	94.0 ± 8.4	0.97 ± 0.11	-	-
26 June	"	65.2 ± 9.2	1.0 ± 0.10	-	-
27 June	"	79.4 ± 12.5	1.1 ± 0.07	-	-
28 June	"	48.2 ± 5.9	1.7 ± 0.17	X	X
2 July	"	81.8 ± 10.2	11.0 ± 2.22	-	-
3 July	"	72.1 ± 7.2	2.1 ± 0.33	X	X
4 July	"	91.7 ± 9.2	1.9 ± 0.38	X	X
5 July	"	39.2 ± 5.1	1.3 ± 0.26	-	-
8 July	"	46.6 ± 4.8	1.6 ± 0.36	X	-
10 July	"	65.3 ± 11.7	2.5 ± 0.81	X	-
11 July	"	70.8 ± 10.8	2.8 ± 0.82	X	-
12 July	"	39.8 ± 4.4	4.5 ± 0.93	X	X
15 July	"	63.3 ± 11.8	1.9 ± 0.44	X	X
16 July	"	90.7 ± 9.3	1.6 ± 0.22	X	X
18 July	"	60.8 ± 5.5	3.9 ± 0.93	X	X
19 July	"	35.9 ± 3.1	1.1 ± 0.13	-	-
22 July	"	65.9 ± 9.9	1.2 ± 0.25	-	-
23 July	"	56.3 ± 3.1	7.5 ± 1.6	X	X
24 July	"	74.0 ± 5.5	3.8 ± 0.41	X	X
25 July	"	48.7 ± 4.0	1.8 ± 0.41	X	X
1 August	"	47.2 ± 3.2	0.85 ± 0.16	-	-
7 August	"	38.7 ± 3.5	1.07 ± 0.16	-	-

background amounts of radiocesium.

The grand mean total-beta activity of Tabanidae samples trapped at the radioactive pond was 4 cpm/insect (Table XX), about a factor of four higher than the activity of horseflies collected at Pinawa. Overnight counting on a gamma spectrometer showed radiocesium accounted for more than 85% of the radioactivity measured in pond horseflies. Thus only a small amount of radiocesium was removed from the radioactive pond by each adult Tabanid. The average weight of all the horseflies caught at this pond in 1968 was about 60 mg. If one assumes that 4 cpm/insect is representative of the mean activity of adult Tabanids which emerged from the radioactive pond, then the average specific activity of newly emerged adults was 67 cpm/g. The counting efficiency of the counter was 30%, therefore the quantity of radioactivity removed would be about 100 pCi/g of insect. In July at the height of the local horsefly season, about 2000 Tabanidae were taken from the trap each day, or 120 g of horseflies. Even if <u>all</u> these insects had emerged from the radioactive pond, they would account for only 0.012 microcuries ^{137}Cs/day, an insignificant fraction (less than 2.5×10^{-8}) of the radiocesium initially added to the radioactive pond. Adult horseflies which lived during their larval stage in an aquatic habitat contaminated with radiocesium could, therefore, be expected to transfer negligible amounts of that nuclide to adjacent land foodchains. If this finding generally applies to other aquatic insect

species, the use of open pits, which have been fenced to keep out large animals, may be a feasible method for disposing low-level radioactive liquid wastes in regions where soil porosity prohibits disposal into the ground. Although radiocesium is a most convenient nuclide to measure, the results of the Tabanid measurements which have been reported show that it is not suited, at least in permissible amounts, to use as an ecological tracer for following the dispersion of adult aquatic insects which have emerged from a labeled aquatic habitat. It is concluded from the field study described that most of the radiocesium which was added to the radioactive pond was sorbed by the pond mud, and less than 5% was retained and transferred through the pond biota. This conclusion agrees with the findings of other workers (Pendleton and Hanson, 1958; Crossley and Shanks, 1967). Had samples of emerging Chironomids for radiochemical analysis been obtained, it might have been possible to show that these insects, because of the specific activity of their larvae, accounted for a significant fraction of the ^{137}Cs not bound up in the radioactive pond mud and algae.

CHAPTER IX

DISTRIBUTION OF RADIATION DOSE IN THE RADIOACTIVE POND

INTRODUCTION

The biological significance of radiocesium accumulation by the biotic and abiotic components of the radioactive pond is the radiation dose delivered to the flora and fauna inhabiting the pond ecosystem. The measurements described in the previous chapter established that most of the ^{137}Cs added to the pond was sorbed by the pond mud, a significant fraction was accumulated by the algae, and a much smaller fraction was taken up by the insect component of the pond community. Because of these findings, it is to be expected that organisms living at the water-mud interface of the radioactive pond would receive the greatest radiation dose. Therefore a method for determining the integrated radiation dose received by these organisms was required.

The calculation of total dose from the nuclide concentration in the water and mud has been a common practice, Nelson and Blaylock (1963), and Pickering et al. (1966). This method requires considerable sampling, and many analyses and calculations, to obtain a realistic estimate of dose distribution. At any early stage of the field work it was appreciated that a technique permitting measurement of radiation does in situ would be advantageous. Blaylock (1966), studying the induction of chromosome aberrations in Chironomidae,

used Toshiba low atomic number metaphosphate glass-rod dosimeters to estimate the dose to larvae living in radioactive mud. Gyllander (1966), has used a $CaSO_4$:Mn type of thermoluminescent dosimeter to follow the diffusion in water of low level radioactive wastes.

A principle of dosimetry is that the device used to measure the dose to an object should be as much like the object in shape, density and atomic number as possible (Fowler and Attix, 1966). Lithium fluoride (LiF) has an atomic number nearly equivalent to that of tissue, thus a LiF dosimeter of comparable volume should give a satisfactory estimate of the dose to an organism such as a Chironomid larva. The principles and techniques of LiF dosimetry have been described by Cameron et al. (1964), Fowler and Attix (1966), and by Worton and Holloway (1966). Ashby et al. (1967) have used the technique to measure radiation dose in a terrestrial habitat. In the work to be described, the application of LiF dosimetry to measurement of radiation dose delivered to benthic organisms living in the radioactive pond was investigated.

METHODS

In 1967 at the beginning of the experiments, the radioactive pond was about 19 m in diameter and increased uniformly in depth to 2 m at the centre. Because of evaporation, the diameter of the pond decreased to 17 m by the end of the summer. In contrast, because of heavy rainfall (Appendix 'C'), the pond diameter increased throughout the summer of 1968.

Dosimeters

Preliminary dosimeter tests were conducted in a 110 litre glass aquarium, which contained a sloping layer of mud taken from the radioactive pond before adding ^{137}Cs to the pond in May 1967. Following the addition of ^{137}Cs to the aquarium water, it was found that 80% of the nuclide was sorbed by the first centimeter of mud.

Two types of dosimeter were tested in the aquarium. One was a cylindrical plastic tube,[1] 20 mm long and 4 mm OD, filled with 47 mg of LiF powder[2] and sealed in place with a plastic plug (Figure 17-A). The other type was a flat disc, 12 mm in diameter, and 0.1 mm thick, LiF powder dispersed in Teflon[1] (Figure 17-C). Both types of dosimeter were stacked 3 mm apart, and the stacks inserted vertically in the aquarium mud to such a depth that the mud-water interface passed through the middle of each stack.

The disc-type dosimeter was unsatisfactory. It soon became mud-stained to an extent that severely affected accuracy of reading, and it adsorbed ^{137}Cs on its surface. The cylindrical dosimeter (Figure 17-A) leaked. Coating it with Dow Corning RTV silicone rubber (Figure 17-B) prevented leakage, and it was the coated dosimeter which was used in the ponds.

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[1]Controls for Radiation Incorporated (ConRad), Cambridge, Mass.

[2]Harshaw Chemical Co., Cleveland, Ohio.

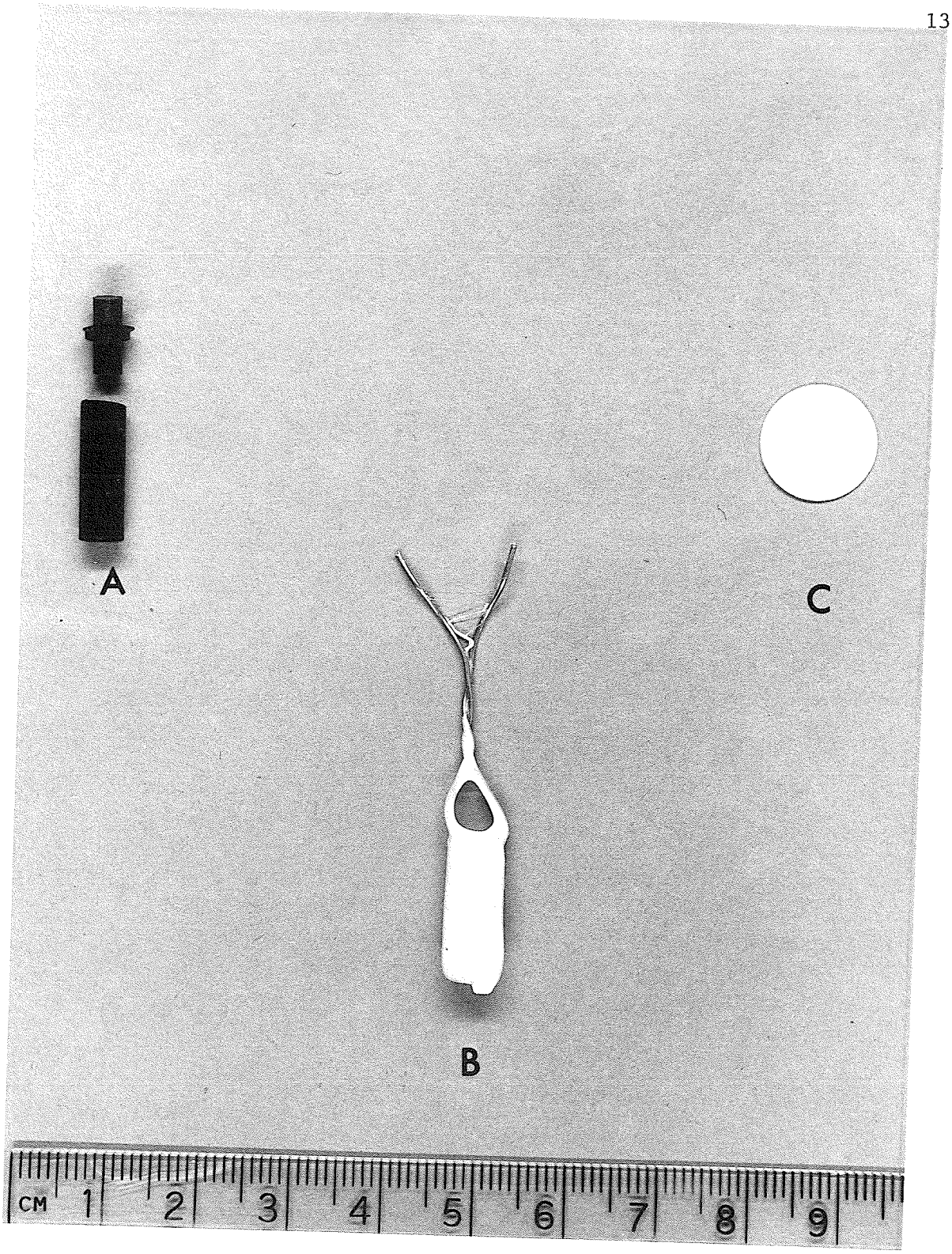

Figure 17

A - cylindrical dosimeter with sealing plug.

B - cylindrical dosimeter, filled with LiF powder, and coated with silicone-rubber water proofing compound.

C - Teflon disc dosimeter.

Placement of Dosimeters in Ponds

The dosimeters were fastened at 1 m intervals on lengths of nylon parachute cord with small pieces of wire (Figure 17-B). These cords or strings, were weighted with 10 g lead weights placed between the dosimeters, to ensure that the strings rested on the bottom of the pond. The preliminary experiments had shown that the dose rate was greatest at the mud-water interface, and that it was reduced by a factor of two at 2.5 cm above the interface. Following the addition of 0.5 Ci of ^{137}Cs to the radioactive pond, the strings were placed in the ponds in such a way that the dosimeters lay at the intersection of a 1 m^2 grid. One hundred and seventy dosimeters were placed in the radioactive pond, and 80 dosimeters were put in the non-radioactive pond.

Two strings of dosimeters were removed from the non-radioactive pond in June and August, 1967, respectively, to check that water had not entered them. Nine strings of dosimeters were withdrawn from the radioactive pond at the end of October, 1967, prior to freeze-up. The remaining strings were taken out of both ponds in May, 1968; they were unaffected by freezing. At the same time strings of new dosimeters were placed in both ponds, and these were removed in October, 1968.

Measurements

The dosimeter powder was read on a ConRad Model 5100 TLD reader. As previously described, samples of pond water and mud were

taken at regular intervals throughout the summer of 1967 and 1968 (Figure 15 and Appendix 'J').

RESULTS AND DISCUSSION

^{137}Cs emits beta particles in addition to gamma photons. In an infinite medium of uniform radioactivity, the beta particles contribute about 30% of the total dose which would be delivered to a small organism (Morgan, 1959). The dosimeters used in the ponds recorded only the gamma component of the radiation dose; they were insensitive to beta particles. However, the preliminary experiments conducted in the aquarium had shown, when the pond dosimeter was compared with the beta-sensitive disc dosimeter (Figure 17-C), that the total dose could be inferred by measuring the gamma component only.

Pond Dosimeter Readings

Each string of dosimeters taken from a pond provided a cross-section of the dose distribution. Each distribution was plotted to scale on cardboard, cut out, then assembled to form a three dimensional model from which an isodose plot of the pond was prepared. The isodose plot representing the radioactive pond measurements in 1967 appears in Figure 18. Aspects of this study have been reported in the literature (Guthrie and Scott, 1969).

The most significant feature of the isodose contours shown in Figure 18 is the non-uniformity of the dose distribution. Compared

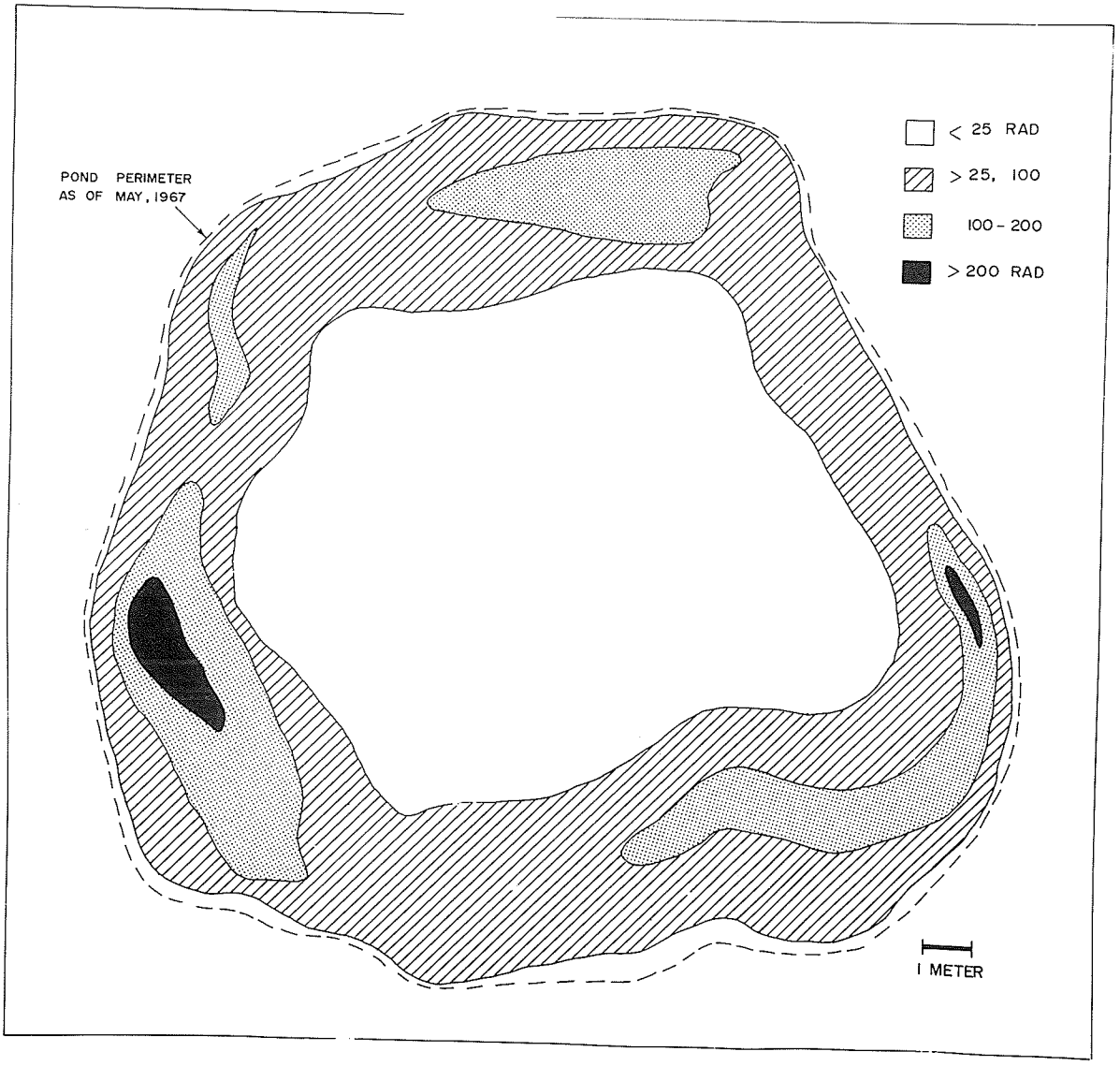

Figure 18

Isodose plot showing distribution of radiation dose in radioactive pond in 1967

to the centre of the pond, a relatively large radiation dose was delivered to benthic organisms living in the vicinity of the pond margin, where in some locations the total dose exceeded 200 Rad. The total dose delivered at the centre of the pond was less than 25 Rad. One string of dosimeters at the radioactive pond was on dry ground, about 2 m from the edge of the pond. The average total dose registered by this string was 400 mRad and it was uniformly distributed. A uniform distribution of dose was also recorded by the readings obtained from the dosimeters placed in the non-radioactive pond, the average reading being 50 mRad. This does is attributed to background radiation. The dose distribution recorded by the dosimeters placed in the radioactive pond during the summer months of 1968 confirmed the dose distribution shown in Figure 18. During the summer of 1967 the water level in the ponds decreased steadily; little rain fell during the period. In contrast the summer of 1968 was a particularly wet one, and the water level in the ponds increased throughout the summer. The fact that the different water level conditions in 1967 and 1968 did not change the overall pattern of dose distribution is an indication that the radiocesium in the radioactive pond was firmly bound to the pond mud, and little exchange with the pond water and biota occurred.

The total-beta activity of the pond mud (Appendix 'J') confirmed the dose distribution measured by the dosimeters. No emergent aquatic plants were growing in the radioactive pond in 1967,

but algae were present that year. Gamma spectrometric analysis of algae samples ruled out the possibility that biological transfer of ^{137}Cs by algae contributed significantly to the non-uniform dose distribution. A physical explanation must be sought. It is possible that transport of radiocesium through the pond mud by capillary action occurred at the pond margin shortly after the nuclide was added to the radioactive pond, and was concentrated there as a result of the water evaporating at the margin.

The non-uniform distribution of dose in the radioactive pond has ecological implications. Chironomids living near the pond margin could be receiving a radiation dose two orders of magnitudes higher than that received by the same insects living closer to the centre. Figure 18 shows that only a small spatial displacement could result in a significantly different total dose. The importance of this finding to the pond Chironomid population is being followed.

It is concluded that LiF dosimetry has been shown to be a practical field method for measuring the total radiation dose in an aquatic habitat. It required less time and effort than did dose distribution estimation by repetitive measuring of the radioactivity in the pond mud and water samples, and required only one measurement to determine the integrated dose at a given location.

CHAPTER X

SUMMARY AND CONCLUSIONS

The uptake and elimination of the radionuclide ^{137}Cs by species of aquatic insects has been studied in the laboratory, and its fate after addition to a small permanent pond investigated.

Giant water bugs, Lethocerus americanus (Leidy) were fed tadpoles labeled with ^{137}Cs (radiocesium). The uptake of this nuclide, and its elimination when the water bugs were subsequently fed unlabeled tadpoles, was measured and expressed in terms of biological half-life (T_b). A biological half-life of 4.5 days was obtained for 4th instar L. americanus, and a T_b = 10.8 for the adults. The finding that adult water bugs eliminated radiocesium at a slower rate than the juvenile stage agrees with comparable observations made by other workers who studied ^{137}Cs elimination by terrestrial insects. A significant decrease in the radiocesium activity of L. americanus nymphs was observed prior to molting, an indication that the insects had ceased to feed. The radionuclide label method, therefore, offers a useful means for anticipating this important physiological event in the field.

A simple two compartment model was developed and used to calculate the assimilation fraction (F), that is the fraction of ingested food which entered the haemocoele of adult water bugs. The value A = 0.07 calculated from the model is small, compared with assimilation fractions which have been determined for adult terrestrial

beetles and isopods by other investigators. Hemipterans do not consume all the prey. Therefore another parameter should be incorporated into the model to compensate for the fact that L. americanus, unlike adult beetles, do not ingest their food completely. The effect of molting and the method of feeding on the amount of ^{137}Cs accumulated, must be allowed for when using this radio-label to determine the food consumption of aquatic insects in natural situations.

The uptake and elimination of ^{137}Cs by Aedes aegypti L., A. atropalpus (Coquillett) has been examined. These studies showed the biological half-life of radiocesium in these species differed from published T_b values obtained for insects of comparable size. This difference is attributed to habitat. Of equal importance, however, was the finding that most of the ^{137}Cs accumulated by A. aegypti larvae entered via diffusion across the papillary membrane, and not by assimilation from the gut. This diffusion process was suppressed by potassium, and is assumed to be the result of competition between K^+ and Cs^+ for membrane diffusion sites. The suppression of ^{137}Cs uptake by potassium was temperature dependent. It is concluded that radiocesium is not a suitable radionuclide with which to label mosquito larval food for determination of food consumption. In an aquatic environment, the label leaches out of the food into the rearing medium and is then accumulated via diffusion across the papillary membrane. Consequently, the essential

premise of the radioactive label technique, that the food consumed is proportional to the amount of label accumulated, no longer holds true.

No radiocesium was accumulated by mosquito pupae as a result of the nuclide penetrating the pupal exuvia. The biological half-life of ^{137}Cs in male and female A. aegypti was 40 hrs. Most of the radiocesium label was eliminated via the fecal pellets. The eggs were only slightly radioactive.

A permanent pond, diameter 19 m, was contaminated with 0.5 Ci of ^{137}Cs. Most of the contaminant was sorbed by the pond-bottom mud, less than 5% being distributed through the biota. The algae growing in the contaminated pond had the highest specific activity (cpm ^{137}Cs/g) of all biota samples. Horseflies (Tabanidae) were trapped at the contaminated pond. The radiocesium activity of these flies, compared with that of horseflies trapped at a distant location, indicated that they had emerged from the margin of the contaminated pond. Chironomid larvae had the highest specific activity of all the insect species analysed. Concentration factors were calculated from these specific activities, and it was found that an insect's primary food source could be traced in this way.

The sorption of the radiocesium contaminant by the pond mud was measured. A simple method utilizing lithium fluoride dosimeters was developed to measure the integrated dose of ionizing radiation delivered to organisms living at the water-mud interface.

It was found that doses as large as 2 rad/day were received in some areas of the pond bottom. It is suggested that this finding might account for the absence from the contaminated pond of certain species of algae, which were abundant in a nearby uncontaminated pond.

It is concluded that the radionuclide label or tracer technique is a valuable adjunct to existing methods for studying foodchain energetics in aquatic habitats.

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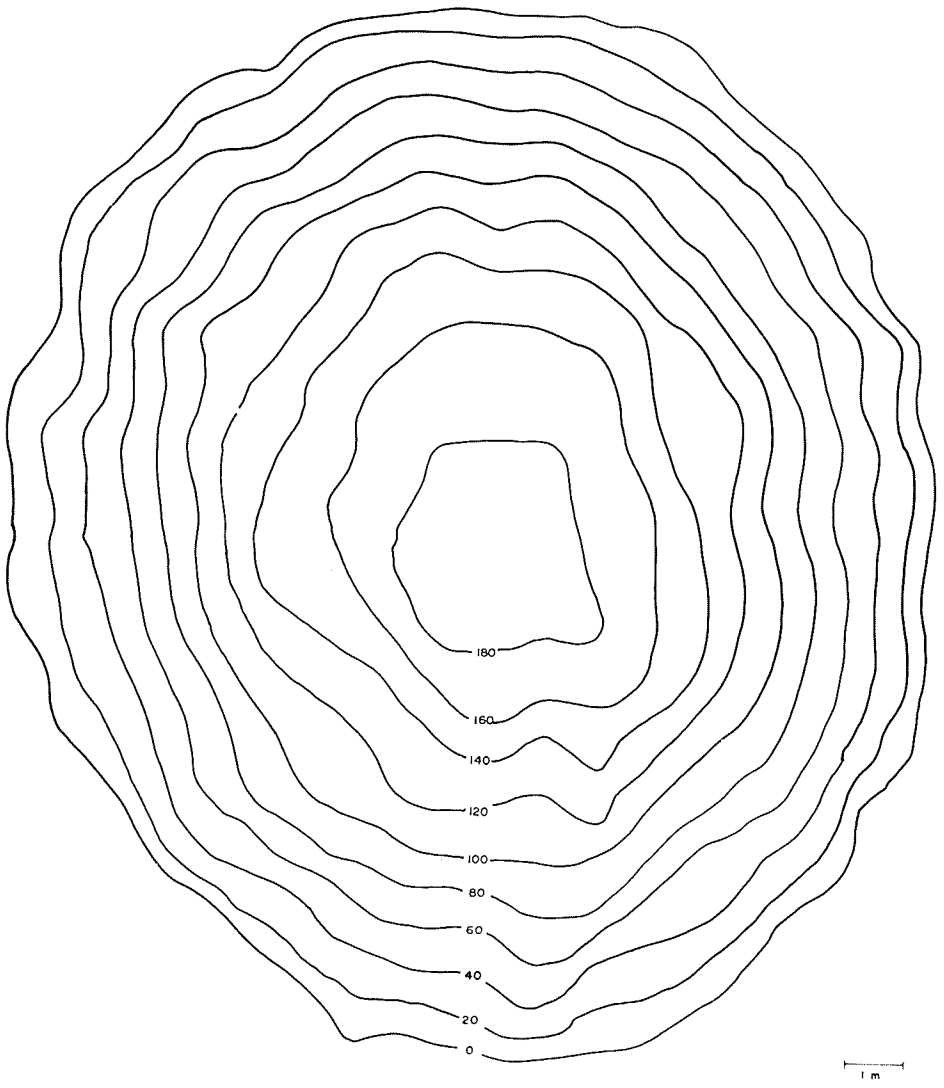
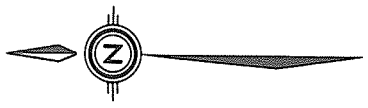
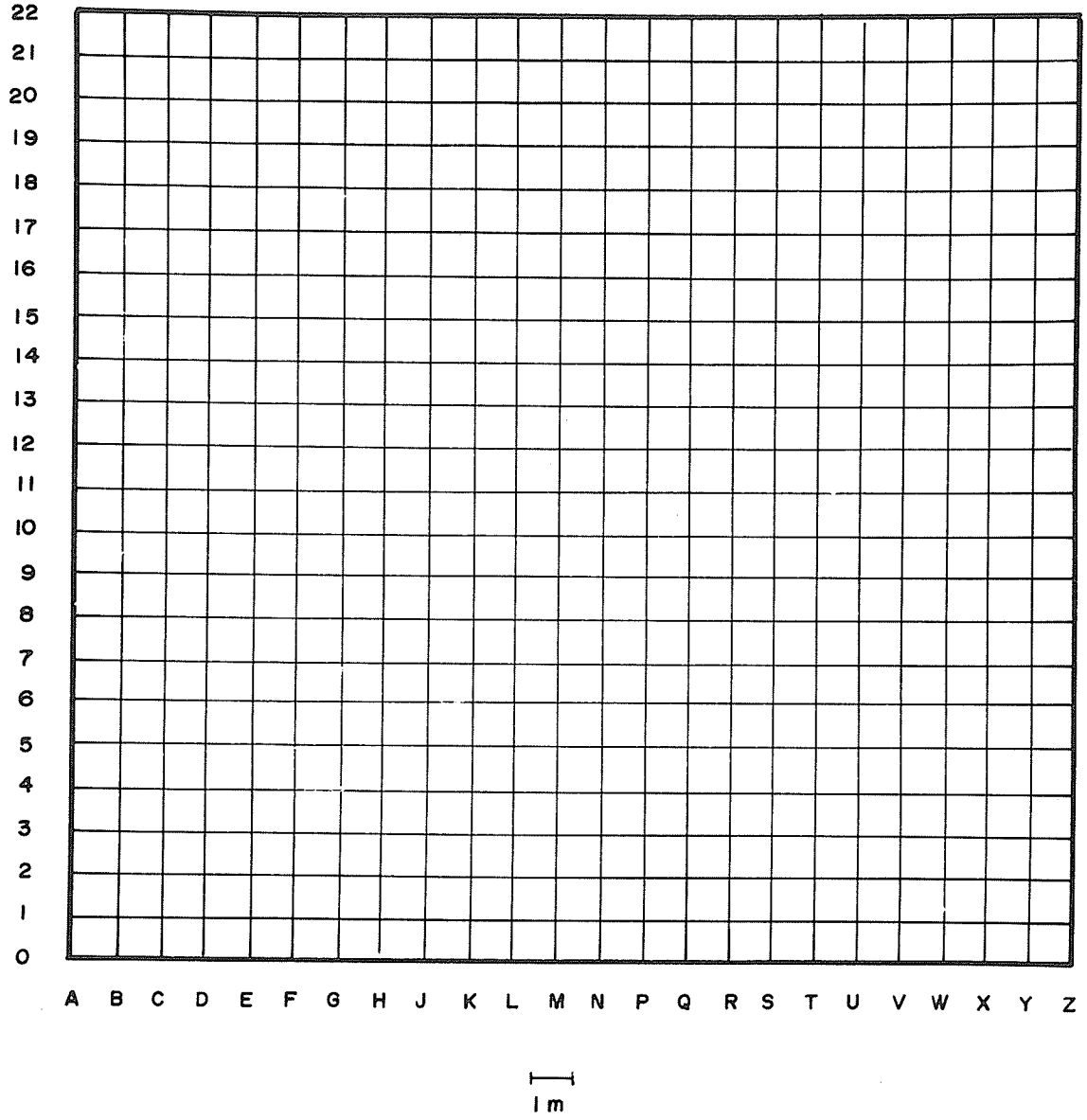
APPENDIX A



Depth contours in cm. of radioactive pond
as measured in June 1967

APPENDIX B





1 m^2 sampling grid used to orientate bottom sampling in radioactive and non-radioactive ponds

APPENDIX C

Summary of Summer Weather in Vicinity of Radioactive Pond - 1967 and 1968

Five day mean air temperature and %-relative humidity measured at 1.5 m above ground

Period	Temperature - °F		Relative humidity	
	1967	1968	1967	1968
April 20-24	-	34.6	-	75.9
April 25-29	-	41.4	-	58.2
April 30-May 4	-	48.7	-	53.6
May 5-9	-	41.5	-	75.5
May 10-14	-	48.5	-	65.1
May 15-19	45.9	33.1	67.4	92.2
May 20-24	50.9	51.7	63.4	52.2
May 25-29	58.3	50.9	62.1	79.9
May 30 - June 3	63.4	56.3	53.3	78.3
June 4-8	51.5	54.3	71.8	86.3
June 9-13	58.9	52.8	72.3	83.3
June 14-18	60.8	53.4	67.9	66.4
June 19-23	51.2	56.1	80.1	69.0
June 24-28	61.1	54.6	71.5	69.4
June 29 - July 3	56.2	no mean	75.7	no mean
July 4-8	62.9	no mean	75.8	no mean
July 9-13	60.0	no mean	73.7	no mean
July 14-18	63.2	no mean	74.2	no mean
July 19-23	70.3	no mean	81.6	no mean
July 24-28	60.6	54.9	77.6	74.9
July 29 - August 2	58.7	58.5	81.9	80.9
August 3-7	59.3	60.8	79.0	87.6
August 8-12	59.2	52.4	75.5	80.7
August 13-17	68.0	48.6	74.6	85.8
August 18-22	56.0	55.4	72.1	93.4
August 23-27	62.2	51.7	68.6	86.0
August 28 - September 1	56.6	58.5	69.4	90.1
September 2-6	67.2	50.2	70.1	89.1
September 7-11	56.9	54.4	72.6	81.8
September 12-16	60.4	65.9	84.5	82.4
September 17-21	55.5	55.9	79.7	88.8
September 22-26	48.1	49.7	70.5	88.5
September 27 - October 1	51.5	49.6	64.7	82.1
October 2-6	41.4	41.9	81.4	83.5

APPENDIX C Cont'd.

Summary of Summer Weather in Vicinity of Radioactive Pond - 1967-1968

Monthly rainfall and hours of sunshine

Month	Rainfall				Hours of sunshine	
	1967		1968		1967	1968
	in.	cm.	in.	cm.		
May	0.20	0.5	3.27	8.3	-	-
June	1.54	3.9	3.88	9.9	108.6	123.5
July	3.18	8.1	5.96	15.1	155.7	187.1
August	1.62	4.1	3.37	8.6	146.8	109.4
September	0.08	0.2	2.78	7.1	118.8	103.9
October	1.57	4.0	1.40	3.6	42.3	-

$\bar{X}$ = arithmetic mean
N_2 = sample size
s^2 = variation

APPENDIX D

Instar Dimensions of LETHOCERUS AMERICANUS (Leidy)

Pinawa, Manitoba

Part length - mm	2nd Instar, N=6		3rd Instar, N=8		4th Instar, N=9		5th Instar, N=9		Adult, N=8	
	$\bar{X}$	s^2	$\bar{X}$	s^2	$\bar{X}$	s^2	$\bar{X}$	s^2	$\bar{X}$	s^2
Body length	14.5	0.500	19.8	1.066	27.9	3.528	38.3	10.000	54.7	13.572
Body width	7.3	0.125	9.8	0.209	14.1	1.111	18.1	1.361	20.5	1.583
Inter-occular distance	1.3	0.125	1.7	0.066	1.9	0.111	2.6	0.173	3.4	0.119
Beak-length-1st segment	0.4		0.8	0.043	0.7	0.021	0.9	0.039	0.9	0.059
Beak-length-2nd segment	0.8	0.010	0.9	0.008	0.5	0.022	0.9	0.075	1.4	0.226
Beak-length-3rd segment	0.8	0.010	0.8	0.035	0.5	0.037	0.8	0.069	0.9	0.096
Beak-length-4th segment	0.4	0.020	0.4	0.046	0.7	0.060	0.9	0.070	1.2	0.071
Forelegs										
Coxa	1.3	0.125	1.6	0.119	2.1	0.048	2.6	0.402	3.6	0.369
Trochanter	1.5	0.500	1.9	0.035	2.1	0.090	2.5	0.187	3.4	0.226
Femur	4.8	0.125	6.0	0.250	7.9	0.465	10.5	0.562	13.9	0.535
Tibia	3.0	0.50	4.6	0.369	5.4	0.527	7.2	0.625	9.6	0.393
Tarsus	1.3	0.125	1.5	0.083	1.7	0.062	1.8	0.069	2.1	0.202
Claw	1.0	0.001	0.9	0.035	1.4	0.173	1.5	0.187	1.5	0.083
Midlegs										
Coxa	1.3	0.125	1.6	0.125	2.1	0.090	2.6	0.298	3.1	0.226
Trochanter	1.3	0.125	1.9	0.053	2.5	0.125	3.4	0.298	4.1	0.559
Femur	4.3	0.125	5.4	0.482	7.2	0.631	8.7	0.437	12.2	0.654
Tibia	3.5	0.001	4.2	0.066	5.5	0.250	6.6	0.527	9.7	0.821
Tarsus	1.8	0.125	2.3	0.071	2.9	0.840	3.3	0.187	4.1	0.441
Claw	1.0	0.001	1.0	0.001	1.1	0.027	1.4	0.111	1.6	0.141

APPENDIX D Cont'd.

Part length - mm	2nd Instar, N=6		3rd Instar, N=8		4th Instar, N=9		5th Instar, N=9		Adult, N=8	
	$\bar{X}$	s^2	$\bar{X}$	s^2	$\bar{X}$	s^2	$\bar{X}$	s^2	$\bar{X}$	s^2
Hindlegs										
Coxa	2.0	0.001	2.1	0.125	2.5	0.125	3.5	0.125	4.1	0.309
Trochanter	1.8	0.125	2.3	0.138	3.0	0.250	3.9	0.111	4.9	0.476
Femur	4.3	0.125	5.7	0.138	7.8	0.562	10.4	0.173	13.4	0.702
Tibia	3.5	0.001	4.8	0.352	6.7	0.256	9.1	0.548	12.8	0.488
Tarsus	3.3	0.125	3.4	0.196	4.6	0.402	5.9	0.736	8.4	0.441
Claw	0.8	0.125	1.1	0.031	1.2	0.131	1.4	0.048	1.8	0.075

$\bar{X}$ = arithmetic mean
N = sample size
s^2 = variation

APPENDIX E

Instar Dimensions of BELOSTOMA FLUMINEUM (Say)
Pinawa, Manitoba

Part length - mm	3rd Instar, N=11		4th Instar, N=6		5th Instar, N=5		Adult, N=8	
	$\bar{X}$	s^2	$\bar{X}$	s^2	$\bar{X}$	s^2	$\bar{X}$	s^2
Body length	10.1	0.704	14.0	0.001	16.4	0.675	22.1	1.339
Body width	5.5	0.322	7.8	0.125	8.5	0.250	10.8	0.214
Inter-occular distance	1.4	0.040	1.8	0.125	1.5	0.001	2.3	0.066
Beak-length, 1st segment	0.7	0.031	0.7	0.045	0.8	0.043	1.1	0.196
Beak-length, 2nd segment	0.6	0.052	1.0	0.001	1.0	0.002	1.8	0.066
Beak-length, 3rd segment	0.6	0.009	1.0	0.020	1.0	0.002	1.5	0.190
Beak-length, 4th segment	0.5	0.032	0.6	0.045	0.6	0.010	0.8	0.138
Forelegs								
Coxa	1.5	0.022	1.8	0.125	1.3	0.200	1.6	0.417
Trochanter	1.4	0.054	1.0	0.001	1.1	0.050	1.6	0.417
Femur	3.5	0.122	3.3	0.125	3.9	0.050	4.9	0.196
Tibia	2.3	0.218	2.3	0.125	2.4	0.050	3.3	0.071
Tarsus	1.2	0.068	1.0	0.001	1.2	0.200	1.3	0.125
Claw	1.0	0.022	0.5	0.001	0.7	0.075	0.8	0.066
Midlegs								
Coxa	1.2	0.068	1.5	0.001	1.4	0.050	1.8	0.066
Trochanter	1.3	0.068	1.5	0.001	1.5	0.001	1.9	0.053
Femur	3.0	0.277	4.0	0.001	4.3	0.075	5.6	0.125
Tibia	2.5	0.172	3.0	0.500	3.5	0.125	4.9	0.053
Tarsus	1.5	0.072	1.5	0.500	1.9	0.062	2.5	0.200
Claw	1.0	0.001	0.8	0.125	0.9	0.062	1.0	0.001

APPENDIX E Cont'd.

Part length - mm	3rd Instar, N=11		4th Instar, N=6		5th Instar, N=5		Adult, N=8	
	$\bar{X}$	s^2	$\bar{X}$	s^2	$\bar{X}$	s^2	$\bar{X}$	s^2
Hindlegs								
Coxa	1.5	0.001	1.8	0.125	1.7	0.075	2.1	0.102
Trochanter	1.4	0.040	2.0	0.001	1.8	0.075	2.3	0.142
Femur	3.4	0.104	5.3	0.125	5.3	0.075	6.7	0.066
Tibia	2.9	0.254	4.8	0.125	4.7	0.325	6.8	0.154
Tarsus	2.5	0.072	2.8	0.125	2.3	0.416	3.3	0.154
Claw	1.0	0.050	1.0	0.001	0.7	0.083	1.1	0.141

APPENDIX F

Vegetation sampled at 12 stations located within a 10 m wide strip surrounding the radioactive pond (Dugle, 1968). X indicates present, and D dominant (See text for details of sampling method)

	SAMPLING STATION												%
	1	2	3	4	5	6	7	8	9	10	11	12	Presence
% ground covered	40	30	30	70	50	50	80	60	70	60	60	40	-
% canopy cover	0	0	0	0	0	0	20	0	0	0	0	0	-
TREES													
Populus balsamifera			X	X	X	X		X		X			50
P. tremuloides	X	X		X	X	X	X	X	X				67
SHRUBS													
Amelanchier sp.						X							8
Cornus rugosa							X						8
Corylus cornuta							X						8
Rosa sp.	X	X					X	X		X			42
Rubus idaeus var strigosus				X					X	X			25
Salix bebbiana	X	X		X	X	X	X	D	D	X			75
S. discolor	X	X	X	D	X	X	X	X	X		X		83
HERBS													
Achillea lanulosa	X	X	X	X	X	X	X	X	X	X	X	X	100
A. sibirica									X				8
Anemone canadensis									X	X			17
Aster sp. unknown		X					X			X			25
Aster ciliolatus											X		8
A. johannensis		X						X					17
A. laevis	X	X					X		X	X	X	X	58
A. simplex					X	X	X	X					33
Bromus sp. #1									X				8
Bromus inermis	X		X	X						D		X	42

APPENDIX F Cont'd.

	SAMPLING STATION												%
	1	2	3	4	5	6	7	8	9	10	11	12	Presence
Carex sp. #2									X	X			17
Carex sp. unknown					X	X			X		X		33
Cicuta maculata									X				8
Cirsium arvense	X	X	X		X			X	X	D	X	X	75
Elymus ? virginicus			X					X	X		X	X	42
Equisetum sylvaticum	X		X	X	X	X		X	X	X	X		75
Erigeron philadelphicum				X									8
Fragaria virginiana	X	X	X	X	X	X	D	X	X	X	X	X	100
Galium septentrionale		X		D	X	X	X	X	X	X			67
Geum aleppicum				X			X	X	X	X	X		50
Grass sp. #1	X	X		X	X			X	X			X	58
Grass sp. #2									X				8
Grass sp. #8		X								X			17
Grass unknown								X					8
Hordeum jubatum	X	X	X		X	X		X		X	X	X	75
Lathyrus ochroleucus		X			X	X	X						33
Medicago lupulina								X					8
M. sativa	D	X			X	X				X	D	D	58
Melilotus albus	X	X	D	X			X	X	X		X	X	75
Scutellaria galericulata										X			8
Phleum pratense	X		X	X	X	X		X	X		X	X	75
Solidago giganteus	D	D	X		D	X	X	X	D	D	X		83
Solidago canadensis				X									8
Solidago graminifolia				X		X		X					25
Sonchus arvensis	D	D	D	D	D	D	X	D	D	D	D	D	100
Stachys palustris				X			X		X	X	X		42
Steironema ciliatum		X						X		X			25
Taraxacum officinale	X			D	X	X	D			X	X	X	67
Trifolium pratense	X	X		X	X	X		X	X	X	X		75
T. upens	X	X	X	X	D	X	X	X		X	X	D	92
Vicia americana	X	X	X	X	X	X	X	X	X	X	X		92

APPENDIX G

Algae in radioactive (R) and non-radioactive (N-R) ponds in 1968. Summary of identifications (Dugle, 1968). ? indicates tentative identification, X indicates present

Division	Class	Order	Family	Genus	Location R	Location N-R
Chlorophyta	Chlorophyceae	Chlorococcales	Characiaceae	Characium spp. #1,2,3,4	X	X
				? Chlorococcus sp.	X	X
			Oöcystaceae	Ankistrodesmus ? braunii (Naeg.) Brunn	X	X
				Ankistrodesmus spp. #2,3,4	X	X
				Closteriopsis	X	-
			Scenedesmaceae	Actinastrum sp.	X	-
				Scenedesmus sp.	-	X
				Tetrallantos sp.	X	-
		Cladophorales	Cladophoraceae	Cladophora sp. #1	X	X
				" #2	X	X
				" #3	-	X
				" #4	X	X
				" #5	X	X
				" #6	-	X
				" #7	X	X

APPENDIX G Cont'd.

Division	Class	Order	Family	Genus	Location R	Location N-R
Chlorophyta	Chlorphyceae	Oedogoniales	Oedogoniaceae	Oedogonium sp.	X	X
		Tetrasporales	Palmellaceae	Gloeocystis sp.	X	-
		Ulotrichales	Ulotrichaceae	? Ulothrix spp. #1,2	X	X
		Volvocales	Chlamydomonadaceae	? Chlamydomonas sp.	X	X
			Haematococcaceae	Haematococcus lacustris (Girod.)	-	X
			Volvocaeae	Pandorina morum Bory	X	X
		Zygnematales	Desmidiaceae	Closterium sp.	X	X
				Cosmarium spp. #1,2	X	X
				Unknown sp. #1	X	-
				Unknown sp. #2	X	-
		Unknown Filamentous Form	?	?	-	X

APPENDIX G Cont'd.

Division	Class	Order	Family	Genus	Location R	Location N-R
Chrysophyta	Bacillariophyceae	Pennales	Unknowns	?	X	X
			Fragilariaceae	Fragilaria sp.	-	X
			Naviculaceae	Calonesis sp.	-	X
				? Hydrosera sp.	X	-
				? Navicula sp.	X	X
	Chrysophyceae	Chrysotrichales	Phaeothamniaceae	Phaeothamnion sp. (epiphytic on Cladophoraceae)	-	X
Cyanophyta	Myxophyceae	Chamaesiphonales	Chamaesiphonaceae	Chamaesiphon sp.	X	X
				Chamaesiphon ? incrustans Grunow	-	X
		Chroococcales	Chroococcaceae	Gloeocapsa ? rupestris Kuetzing	-	X
		Uknown Colonial-Red Colored	?	?	X	-

APPENDIX G Cont'd.

Division	Class	Order	Family	Genus	Location R	Location N-R
Cyanophyta	Myxophyceae	Oscillatoriales	Nostocaceae	? *Anabaena* sp. #1	-	X
				? *Anabaena* sp. #2,3	X	X
				? *Navicula* sp.	-	X
				? *Nostoc* sp. #1	X	X
				? *Nostoc* sp. #2	-	X
			Oscillatoriaceae	*Arthrospira* sp.	X	-
				Lyngbya sp.	X	-
				Oscillatoria sp.-epiphytic* (* very few compared to non-radioactive pond)	X	X
				Spirulina major Kütz	-	X
Euglenophyta	Euglenophyceae	Euglenales	Euglenaceae	? *Euglena* sp. (* very few compared to non-radioactive pond)	X*	X
				Lepocinclis actuta Presc.	X	-

APPENDIX H

Microscopic animals in radioactive (R) and non-radioactive (N-R) ponds in 1968. Summary of identifications. ? indicates tentative identification, X indicates present

Phylum	Class	Order	Family	Genus	Location R	Location N-R
Arthropoda	Crustacea	Cladocera	? Macrothricidae	-	X	X
Protoza	Ciliata	Spirotrichida	Stentoridae	Stentor polymorphus (Dugle, 1968)	-	X
		Holotrichida	Holophryidae	Lacrymaria sp. (Dugle, 1968)	-	X
			Paramecidae	? Paramecium sp.	X	X
		Unknown veriform-wiggler	?	?	X	X
	Large, unidentified	?	?	?	X	X
Tardigrada Found only in non-radioactive pond, very plentiful in algae	?	?	?	?	-	X

APPENDIX I

Aquatic insects collected from the experimental ponds in 1967 and 1968. Major collecting effort was on Hemiptera. ? indicates a tentative identification

Order	Family	Genus	Species
Coleoptera			
	Dytiscidae		
		Acilius	A. semisulcatus (Aubé)
		Colymbetes	C. sculptilis (Harris)
		Dytiscus	D. cordieri Aubé
			D. fasciventris Say
			D. dauricus Gebl.
		Ilybius	I. fraterculus Lec.
		Rhantus	R. frontalis Marsh
	Gyrinidae		
		Gyrinus sp.	
Diptera			
	Ceratopogonidae		
	Chironomidae		
		Glyptotendipes	
		Polypedilum	
		Psectrotanypus	
		Acricotopus	

APPENDIX I Cont'd.

Order	Family	Genus	Species
Ephemeroptera	Baetidae (Ireland, 1968b)	Baetis	
		Caenis	C. ? simulians
Hemiptera	Belostomatidae	Lethocerus	L. americanus (Leidy)
	Corixidae	Cymatia	C. ? americana Hussy
		? Trichocorixa	
	Gerridae	Gerris	G. dissortis Drake and Harris
		? Metrobates	G. ? remigis Say
	Nepidae	Ranatra	R. fusca Palisot de Beauvois
	Notonectidae	Notonecta	N. undulata Say
			N. ? borealis Bueno and Hussey
Odonata	Libellulidae	Libellula L.	
	Gomphidae (Ireland, 1968b)	Opiogomphus	O. ? colubrinus (Say)

APPENDIX J

Total-beta activity, as ^{40}K equivalent, of radioactive pond sediment samples taken in 1967 and 1968. Activity expressed in CPM/g $\pm$ standard error

Month sampled	Location	Activity
May 1967	C-13	$1.15 \times 10^4 \pm 322$
	F-17	$9.70 \times 10^3 \pm 335$
	H- 7	$1.19 \times 10^3 \pm 20$
	J-16	$6.04 \times 10^4 \pm 1583$
	M- 6	$8.31 \times 10^3 \pm 498$
	N- 9	$2.41 \times 10^2 \pm 5$
	P- 7	$5.89 \times 10^2 \pm 28$
	Q-17	$6.53 \times 10^4 \pm 2230$
	R-10	$8.17 \times 10^2 \pm 30$
	S-15	$2.06 \times 10^4 \pm 731$
June 1967	D- 8	$1.55 \times 10^5 \pm 10,178$
	P-10	$2.09 \times 10^3 \pm 93$
	P-15	$6.86 \times 10^3 \pm 692$
	Q- 8	$2.92 \times 10^3 \pm 337$
	R- 9	$1.52 \times 10^3 \pm 173$
	S- 5	$3.01 \times 10^4 \pm 4356$
	S-12	$1.46 \times 10^5 \pm 6664$
	V- 8	$1.84 \times 10^4 \pm 1301$
	V-10	$1.85 \times 10^4 \pm 906$
July 1967	F- 6	$6.52 \times 10^4 \pm 1718$
	F-14	$2.22 \times 10^4 \pm 1419$
	G- 6	$7.86 \times 10^3 \pm 427$
	K- 5	$2.28 \times 10^3 \pm 116$
	K-13	$7.22 \times 10^3 \pm 265$
	L- 5	$1.90 \times 10^3 \pm 75$
	M-15	$1.07 \times 10^5 \pm 4089$
	M-16	$4.60 \times 10^4 \pm 2685$
	P- 9	$8.75 \times 10^2 \pm 76$
	U- 3	$3.00 \times 10^4 \pm 1932$

APPENDIX J Cont'd.

Month sampled	Location	Activity
September 1967	F- 8	$8.63 \times 10^3 \pm 338$
	K- 7	$2.46 \times 10^3 \pm 40$
	L-15	$3.74 \times 10^4 \pm 1439$
	M- 5	$5.28 \times 10^3 \pm 105$
	M- 7	$1.27 \times 10^3 \pm 12$
	N- 8	$2.28 \times 10^3 \pm 140$
	N-10	$2.60 \times 10^3 \pm 110$
	P-12	$2.53 \times 10^3 \pm 3$
	Q- 7	$4.20 \times 10^3 \pm 273$
	U- 9	$3.13 \times 10^4 \pm 253$
October 1967	H-12	$2.92 \times 10^3 \pm 277$
	J- 6	$3.05 \times 10^3 \pm 146$
	J- 7	$9.50 \times 10^2 \pm 43$
	J- 8	$9.34 \times 10^2 \pm 23$
	J-12	$1.78 \times 10^3 \pm 28$
	J-14	$2.22 \times 10^3 \pm 164$
	N-14	$1.47 \times 10^3 \pm 24$
	P- 6	$8.83 \times 10^3 \pm 117$
	R-11	$2.59 \times 10^3 \pm 71$
	R-14	$3.00 \times 10^3 \pm 170$
May 1968	D- 8	$1.41 \times 10^4 \pm 558$
	D-11	$2.22 \times 10^3 \pm 117$
	G- 5	$9.36 \times 10^3 \pm 497$
	J- 8	$2.97 \times 10^3 \pm 288$
	K- 7	$1.89 \times 10^3 \pm 89$
	L- 6	$2.15 \times 10^3 \pm 105$
	N- 9	$4.74 \times 10^3 \pm 23$
	P- 5	$9.89 \times 10^3 \pm 450$
	P- 7	$1.48 \times 10^3 \pm 101$
	R-13	$2.03 \times 10^3 \pm 45$
June 1968	E-11	$5.12 \times 10^3 \pm 115$
	G-12	$8.72 \times 10^3 \pm 403$
	H-12	$2.24 \times 10^3 \pm 144$
	L-16	$3.00 \times 10^4 \pm 827$
	N- 4	$2.88 \times 10^3 \pm 36$
	P-13	$9.39 \times 10^3 \pm 208$
	R-16	$6.90 \times 10^2 \pm 45$
	S- 7	$8.42 \times 10^3 \pm 184$
	T- 7	$5.87 \times 10^3 \pm 382$
	T- 9	$1.28 \times 10^4 \pm 479$

APPENDIX J Cont'd.

Month sampled	Location	Activity
July 1968		
	F-10	$9.30 \times 10^3 \pm 721$
	F-16	$1.26 \times 10^3 \pm 5$
	H-11	$1.67 \times 10^3 \pm 161$
	M- 3	$2.79 \times 10^3 \pm 189$
	P- 8	$4.31 \times 10^3 \pm 95$
	P-10	$3.96 \times 10^3 \pm 23$
	Q-10	$2.14 \times 10^3 \pm 55$
	Q-16	$1.89 \times 10^3 \pm 136$
	R- 7	$1.57 \times 10^3 \pm 77$
	S- 9	$1.67 \times 10^3 \pm 126$
October 1968		
	D- 9	$1.12 \times 10^5 \pm 5963$
	E-10	$2.33 \times 10^4 \pm 1437$
	G- 4	$2.13 \times 10^4 \pm 1373$
	G- 9	$1.67 \times 10^3 \pm 141$
	M-16	$4.87 \times 10^4 \pm 4278$
	Q-13	$7.03 \times 10^3 \pm 273$
	R- 9	$1.59 \times 10^3 \pm 151$
	U- 6	$4.06 \times 10^4 \pm 1795$
	U-11	$9.61 \times 10^3 \pm 770$
	U-16	$4.29 \times 10^3 \pm 33$