PREDICTIVE CAPABILITIES OF THE SPECIFIC ACTIVITY HYPOTHESIS FOR Cs AND Zn IN FRESHWATER SYSTEMS

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#### ABSTRACT

# PREDICTIVE CAPABILITIES OF THE SPECIFIC ACTIVITY HYPOTHESIS FOR Cs AND Zn IN FRESHWATER SYSTEMS

bу

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Predictions of radioisotope concentrations in components of aquatic systems have been attempted using the specific activity concept, an approach that seems theoretically sound. A comprehensive examination of the specific activities of  $^{134}\text{Cs}$  and  $^{65}\text{Zn}$  in the components of a freshwater system, over a 10 month period, was conducted to evaluate the specific activity hypothesis under applied conditions. This study was designed to provide comparisons of predicted and observed specific activities and to test the equivalence of specific activities between all components of the system.

Four ponds were used. A prediction of specific activities was made using Pond 1 data. The distribution of stable Cs and Zn throughout the system was determined and predictions of specific activities, based on these distributions were made. The other three ponds served as controls or provided observed specific activities. Radioisotopes of Cs and Zn were added to the system and after a period of equilibration comparisons were made between predicted and observed specific activities in the water, zooplankton, fish, plants, and sediment.

In all cases observed specific activities for both  $^{134}\mathrm{Cs}$  and  $^{65}\mathrm{Zn}$  were significantly different between components of

the systems at the end of the study period. The results of comparisons of predicted and observed specific activities from Pond 1 are as follows: predicted and observed  $^{134}\text{Cs}$  specific activities for water, zooplankton and fish were not significantly different, while predicted and observed specific activities were significantly different for plants and sediment. Water and fish predicted and observed  $^{65}\text{Zn}$  specific activities were not significantly different while the value for zooplankton, plants and sediment were significantly different.

Predicted <sup>134</sup>Cs and <sup>65</sup>Zn concentrations were lower than observed concentrations, for fish from Pond 1. This non-conservativeness is important where hazard assessments are being made.

One dose of radioisotopes was added to the system in this study and even after 10 months these radioisotopes were not distributed similarly to the stable isotopes. This suggests that the time necessary to reach a specific activity equilibrium might be a matter of years rather than months. More importantly, in natural systems, where the radioisotope addition is continuous, a specific activity equilibrium may never be achieved. These things plus the non-conservative nature of the \$13^4\$Cs and \$65\$Zn predicted concentrations indicates that the use of the specific activity concept for predicting radioisotope concentrations of Cs and Zn in freshwater systems is questionable. A more rigorous approach must be used, considering isotope transfer rates between components and the complexity of the system.

James G. Seelye

Problems with statistical comparisons of derived variables, such as specific activities, are discussed and were considered in interpreting the results of this study.

# PREDICTIVE CAPABILITIES OF THE SPECIFIC ACTIVITY HYPOTHESIS FOR Cs AND Zn IN FRESHWATER SYSTEMS

Ву

James Glenn Seelye

#### A DISSERTATION

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#### INTRODUCTION

Man has identified 105 elements, 90 occur naturally, and 15 are man-made by fission, fusion or activation processes. These 105 elements have 1,934 isotopes, with 1,731 or almost 90 percent being radioisotopes. Only 68 or 4 percent of these radioisotopes occur naturally (Holden and Walker, 1972). The large number of man-made radioisotopes suggests that man has had considerable effect on the isotopic structure of our environment, however most of the man-made radioisotopes are short lived and only relatively small quantities have been produced. The most abundant natural radioisotopes are 238U,  $^{232}$ Th and  $^{40}$ K. Over 90 percent of this natural radiation has been attributed to  ${}^{40}{\rm K}$  (Rice and Duke, 1969). Man Possesses the potential to produce and release large amounts Of radioisotopes into the biosphere through the use of Various nuclear devices. Because of this capability man must understand the ramifications of environmental radioactivity in order to predict and prevent detrimental effects of radioactivity to the earth's biota. Radioecologists have endeavored to describe the cycling of radioisotopes in the environment in order to identify the eventual fates of these materials. Using radioisotopes as tracers and tags have provided a great deal of valuable information on a broad spectrum of ecological processes (Auerbach, 1971).

A radioecological concept that is based on physiochemical similarities of radioisotopes and stable isotopes
is the specific activity hypothesis. This hypothesis was
first proposed by the National Academy of Sciences (1960).
The definition of the term specific activity takes many
forms (Glasstone, 1958); in radioecology it has been defined
as the ratio of radioisotope concentration to total isotope
concentration for a given element. In this work units are
expressed as nCi/mg. The simple two compartment model of
the specific activity concept can be expressed as follows:

$$RI_a = \frac{RI_b \cdot TI_a}{TI_b}$$

Where:  $RI_a$  = Radioisotope concentration in component a

 ${\rm RI_b}$  = Radioisotope concentration in component b

 ${\rm TI}_{\rm a}$  = Total isotope concentration in component a

 ${\rm TI_b}$  = Total isotope concentration in component b

The validity of this relationship rests on the following assumptions:

- 1. There can be no isotope fractionation, the radioisotopes and stable isotopes must exhibit the same physio-chemical behavior.
- 2. All isotopes of the element must be equally available to all components of the system.
- 3. The components of the system must be in equilibrium with respect to the isotopes in question.
- 4. The amount of isotope to be added to the system must be accurately known.

5. Corrections for physical decay of the radioisotopes must be made.

The use of the specific activity concept has been suggested as a method of predicting radioisotope concentrations in the components of ecosystems (Nelson and Kaye, 1971). Supporting evidence for the specific activity concept presented by Nelson (1967a, 1967b) shows the equivalence of the specific activities of  $^{137}\text{Cs}$  and  $^{90}\text{Sr}$  between fish and water. Two species of fish were shown to have similar  $^{137}\text{cs}$  specific activities (Seelye, 1971). Concentration factors for total and radioactive Zn were shown to be similar in oyster flesh by Preston (1967). This similarity allowed prediction of  $^{65}\text{Zn}$  concentrations which agreed favorably with observed values.

Specific activities of <sup>65</sup>Zn in components of an artificial marine community showed large differences on specific activities after a short period of equilibration, indicating a steady-state condition had not been achieved (Duke, 1963). Eyman (1972) found considerable variation in specific activities of <sup>137</sup>Cs in components of a hypereutrophic lake. Data collected from a Columbia River estuary provides <sup>65</sup>Zn specific activities that are different for the components of the system (Renfro, 1968). Specific activities of <sup>137</sup>Cs for a number of fish species in White Oak Lake are given by Kolehmainen (1971) who concluded that because of the apparent differences in these values the entire community is not exhibiting a steady-state distribution of Cs isotopes.

The discrepancies in equivalence of specific activities in aquatic systems are certainly involved with violations of the criteria listed previously. Chemically the isotopes of Cs and Zn should not exhibit isotope fractionation (Bowen, 1966).

There are really two equilibrium components to be considered when discussing specific activities (Patten, 1966). The total element (stable + radioisotopes) distribution can be in equilibrium even while the specific activities of the components have not achieved equilibrium conditions. Patten infers that the rate of change of the specific activities of the components will determine how quickly the stable and radioisotopes achieve identical distributions.

Considering the importance of this concept and because of the conflicting results reported, this study was designed to evaluate the predictive capabilities of the specific activity hypothesis for  $^{134}\text{Cs}$  and  $^{65}\text{Zn}$ .

The Cs radioisotopes are produced as fission products, are long lived and are non-essential, mimicking K in terms of physio-chemical behavior. Zinc is an essential micronutrient and  $^{65}{\rm Zn}$  is produced in relatively large quantities by activation of stable Zn in reactor materials or cooling water. Both  $^{134}{\rm Cs}$  and  $^{65}{\rm Zn}$  are gamma emitters.

The major objective of this study was to compare observed and predicted specific activities of  $^{134}\text{Cs}$  and  $^{65}\text{Zn}$  in a freshwater system, and hence, to evaluate the predictive capabilities of the specific activity hypothesis.

#### METHODS AND MATERIALS

#### Study Site

Four ponds of the Michigan State University, Department of Fisheries and Wildlife Limnological Research Facility were used in this experiment. These ponds are rectangular concrete tanks, 10.7 m by 5.5 m and filled to a depth of approximately 1.2 m with well water. A 10 cm layer of sandy clay loam soil (5 percent organic) was placed in all four ponds. Sediment and water were added and allowed to equilibrate for 23 months before isotope additions and sampling was initiated. The biota of the ponds was allowed to establish naturally except for the fish. Potamogeton crispus and Elodea canadensis were the dominant macrophytes, Ceratium sp. was the dominant phytoplankton and Oedogonium sp. and Spirogyra sp. made up the bulk of the filamentous periphyton during the study period. The zooplankton were separated into four major groups; Order Cladocera, mainly Chydorus sphaericus, subclass Copepoda, naupulii of the two previous groups and Rotifera. Hybrid sunfish Lepomis macrochirus (male) x Lepomis cyanellus (female) of the same age were stocked at a density of 200 fish per pond. Growth rates of these fish were approximately one-third the rates reported by Janssen (1974) for the same hybrid cross but at a much lower density. Benthic macroinvertebrates consisted mainly of

<u>Chironomus</u> spp. and <u>Psectrotanypos</u> spp. in all four ponds. Water chemistry data are given in Tables 1 through 4.

The ponds were originally established in late August 1971 in preparation for this study. Spiking and sampling were initiated in August 1973. During the winter months, November to March 1973 and during the study period from November to May 1974, the ponds were enclosed in a temporary polyethylene greenhouse structure. This structure was equipped with a forced air furnace to maintain air temperatures of 1 to 3° C over the pond water. This eliminated ice cover and facilitated the sampling during the experiment.

#### Isotope Additions

The spiking schedule was designed to allow a prediction of specific activities in one pond, while providing observed specific activities in another pond during the same time period. The experiment was divided into two periods, each four months long. During the first period, starting August 16, 1973, two ponds were spiked. Pond 1 received only stable Cs and Zn and Pond 3 received both stable and radioisotopes of Cs and Zn. The prediction of specific activities was made on the four months data describing the distribution of stable Cs and Zn in Pond 1. These predicted specific activities were then compared with the observed specific activities from Ponds 1, 3 and 4. Ponds 2 and 4 served as controls for the first four months of the study to evaluate any effect of the isotope additions. At the beginning of the second four month period, January 21, 1974, Pond 4 received both

Table 1. Water chemistry data, Pond 1.

Date	Temperature	Hď	Hardness mg/kg CaCO <sub>3</sub>	Alkalinity mg/kg CaCO <sub>3</sub> PHTH. M.O.	nity CaCO <sub>3</sub> M.O.	Total Solids mg/kg	Dissolved Oxygen Diurnal Range mg/kg
08/14/73	21.2	8.6	132.0	0.6	158.0	293.2	4.5-8.5
09/04/73	22.0	7.6	157.0	0.0	169.0	475.0	7.6-10.2
09/25/73	17.0	7.8	156.0	0.0	170.0	250.0	6.2-7.0
10/16/73	11.5	7.9	162.0	0.0	172.0	200.0	8.2-8.5
11/06/73	5.5	8.1	172.0	0.0	168.0	255.0	10.5-11.5
11/27/73	8.0	7.8	168.0	0.0	154.0	245.0	10.4-11.0
12/17/73	0.4	8.0	178.0	0.0	168.0	240.0	11.4-12.2
01/02/74	0.4	9.8	120.0	12.0	118.0	268.0	* 1
02/15/74	0.9	9.1	112.0	28.0	0.46	273.0	ı
03/07/74	0.6	8.7	110.0	26.0	0.96	278.0	9.8-14.4
03/28/74	10.0	9.1	110.0	10.0	130.0	258.0	8.0-12.7
04/18/74	13.5	0.6	112.0	32.0	106.0	318.0	9.5-15.3
05/07/74	12.5	8.9	110.0	30.0	108.0	288.0	9.2-16.4

\*Samples were not analyzed.

Table 2. Water chemistry data, Pond 2.

Date	Temperature °C	Нď	Hardness mg/kg CaCO <sub>3</sub>	Alkal mg/kg PHTH.	Alkalinity mg/kg CaCO <sub>3</sub> PHTH. M.O.	Total Solids mg/kg	Dissolved Oxygen Diurnal Range mg/kg
08/14/73	22.0	0.6	108.0	20.0	133.0	280.0	7.2-11.0
09/04/73	22.5	7.6	124.0	0.0	131.0	* 1	6.8-9.8
09/25/73	17.0	7.8	138.0	0.0	142.0	ı	6.1-6.5
10/16/73	12.5	7.2	152.0	0.0	166.0	ı	5.8-6.5
11/06/73	5.5	7.7	176.0	0.0	160.0	1	11.0-11.6
11/27/73	8.0	7.7	164.0	0.0	160.0	ı	9.4-10.0
12/11/73	4.0	7.6	172.0	0.0	190.0	ı	11.8-12.7
01/02/74	0.9	8.7	122.0	14.0	118.0	220.0	1
02/15/74	0.9	8.7	122.0	16.0	114.0	180.0	11.0-15.0
03/07/74	8.5	8.9	110.0	32.0	102.0	ı	6.2-11.1
03/28/74	10.0	4.6	108.0	20.0	120.0	1	8.0-13.1
04/18/74	14.0	0.6	106.0	30.0	98.0	1	8.8-14.8
05/07/74	13.5	0.6	106.0	32.0	100.0	242.0	8.8-15.7

\*Samples were not analyzed.

Table 3. Water chemistry data, Pond 3.

Date	Temperature °C	Hd	Hardness mg/kg CaCO <sub>3</sub>	Alkalinity mg/kg CaCO <sub>3</sub> PHTH. M.O.	inity caco <sub>3</sub> M.O.	Total Solids mg/kg	Dissolved Oxygen Diurnal Range mg/kg
08/14/73	21.2	9.6	110.0	31.0	123.0	360.0	7.0-11.2
09/04/73	21.0	8.2	122.0	0.0	132.0	300.0	8.0-9.5
09/25/73	17.0	8.2	142.0	0.0	144.0	250.0	6.7 - 7.5
10/16/73	12.0	8.2	130.0	0.0	144.0	200.0	7.8-9.0
11/06/73	5.0	8.2	154.0	0.0	160.0	290.0	12.3-13.5
11/27/73	8.0	8.0	154.0	0.0	150.0	215.0	10.1-10.8
12/17/73	4.0	8.0	166.0	0.0	170.0	* 1	i
01/05/74	5.5	8.5	118.0	10.0	116.0	177.0	1
02/15/74	5.5	8.9	114.0	20.0	112.0	200.0	11.5-15.3
03/07/74	0.6	8.7	108.0	28.0	100.0	255.0	9.8-13.8
03/28/74	10.0	4.6	108.0	20.0	120.0	225.0	ı
04/18/74	13.0	8.9	110.0	28.0	102.0	262.0	9.1-15.0
05/07/74	14.0	9.5	108.0	38.0	0.96	218.0	8.0-15.5

\*Samples were not analyzed.

Table 4. Water chemistry data, Pond 4.

Date	Temperature	Нď	Hardness mg/kg CaCO <sub>3</sub>	Alkalinity mg/kg CaCO PHTH, M.O	inity caco <sub>3</sub> M.O.	Total Solids mg/kg	Dissolved Oxygen Diurnal Range mg/kg
08/14/73	21.5	8 8	143.0	14.0	167.0	307.0	7.9-10.5
09/04/73	22.0	8.2	140.0	0.0	155.0	* 1	7.6-9.5
09/25/73	17.0	7.8	150.0	0.0	166.0	ı	7.6-8.7
10/16/73	13.0	7.6	154.0	0.0	178.0	ı	7.6-8.2
11/06/73	5.5	7.9	168.0	0.0	162.0	ı	11.0-11.6
11/27/73	8.0	7.8	168.0	0.0	164.0	ı	9.3-10.4
12/11/73	4.0	7.8	170.0	0.0	180.0	ı	1
01/02/74	5.5	8.9	124.0	20.0	120.0	120.0	ı
02/15/74	0.9	8.7	122.0	16.0	114.0	240.0	ı
03/07/74	8.5	0.6	106.0	30.0	98.0	250.0	8.8-13.5
03/28/74	10.0	9.5	112.0	12.0	132.0	322.0	9.8-14.7
04/18/74	14.0	9.1	108.0	36.0	100.0	270.0	9.4-15.5
05/07/74	12.0	7.6	110.0	36.0	100.0	292.0	9.0-17.0

\*Samples were not analyzed.

stable and radioactive Cs and Zn, while Pond 1 received  $^{134}\mathrm{Cs}$  and  $^{65}\mathrm{Zn}$  whose distribution after four months had been predicted. This design provides for a comparison between predicted specific activities from Pond 1 and observed values from all three ponds spiked.

Three ponds were spiked with 3 mCi each of 134Cs and  $^{65}{
m Zn}$  obtained from Amersham/Searle Corporation. Stable CsCl and ZnCl<sub>2</sub> were added (7.134 g) to each of three ponds to provide initial concentration in the water of approximately 0.1 mg/kg. These concentrations were sufficient to facilitate analyses, but not high enough to affect the biota, as will be shown. The spiking was accomplished using an 80 liter polyethylene container, calibrated in 10 liter intervals. This container was filled with pond water and all spiking materials. The solution was sprayed onto the pond, one quadrant at a time, to insure an even distribution. Spiking was done on a windy day to promote thorough mixing. Water samples were taken three days after spiking and counted for gamma activity to determine the homogeneity of the spike in the water. From these data (<5 percent variation between samples) it appeared that thorough mixing of the spike had occurred in all three ponds.

# Statistical Methods

When using derived variables based on two independent variables for statistical comparisons, the relative inac-curacy of ratios due to the combination of the error from both independent variables is a problem. Another drawback

of ratios is that the distributions may be unusual. This may be true even where both of the independent variables were normally distributed. Often this problem can be overcome by appropriate transformations. In the case of specific activities measured in this study, square root, logrithmic and arcsin transformations, failed to compensate for heterogenous variances. One-way analysis of variance, with an approximate F test (Box, 1954) was used to adjust the degrees of freedom for the critical F values where heterogenous variance was present.

### Sampling, Sample Preparation and Analysis

Samples of water, zooplankton and fish were taken at 21-day intervals throughout the study period. Sediment and primary producers were sampled at the beginning, middle and end of the study period. Five replicate samples were taken of each component at every sampling time.

Quantitative analysis of total Cs and Zn was accomplished by flame emission and atomic absorption respectively. A dual-channel instrument was used with an air-acetylene flame and wavelength of 2138.6 Å for Zn and air-hydrogen flame and a wavelength of 8521 Å for Cs. All samples were in a nitric acid solution for analysis and appropriate standards, encompassing the range of concentrations encountered, were used.

Determination of radioisotope concentrations were performed using a 512 channel spectrometer equipped with a sodium iodide, thallium activated detector. All samples were counted for  $^{134}\mathrm{Cs}$  and  $^{65}\mathrm{Zn}$  activities with constant geometry,

compared with calibrated standards and corrected for physical decay to the date the activities of the radioisotopes used in spiking were measured. Counting times sufficient to give 95 percent statistical reliability were used in counting all samples (Seelye, 1974).

#### Water

Water samples were collected using a plastic VanDorn water bottle. During each sampling period, five replicate samples were taken randomly with respect to depth and location within each pond. A portion of each water sample was centrifuged and counted to determine radioisotope concentrations. Centrifugation was used to remove particulate solids rather than filtration to eliminate any changes in the concentrations of Cs and Zn caused by the materials used in filtration procedures. A portion of the centrifuged water was then freeze-dried to preconcentrate the isotopes by a factor of 10 to 20 times to facilitate the Atomic Absorption analyses for total Cs and Zn.

Basic water chemistry measurements, alkalinity, hardness, total solids and dissolved oxygen were determined using methods described in Standard Methods (1971). Measurements of pH were made using a line operated pH meter. Diurnal oxygen measurements were recorded using an oxygen-temperature monitor. These data were used to estimate gross primary productivity (McConnell, 1962).

#### Zooplankton

Total seston samples were collected by pumping water through 7  $\mu$  mesh nylon bloting cloth at a rate of 19.0 liters per minute. Sufficient amounts of material were collected in five minutes under normal conditions. In extreme conditions of high or low standing crops of plankton two or ten minute samples were collected. Samples were collected between two to four hours after dark, from five randomly chosen locations in each pond, at a depth of 20 to 30 cm. The samples were collected in 100 ml plastic vials and allowed to settle overnight at 5° C.

Separation of living zooplankton from the rest of the seston was accomplished by aspirating the living, freeswimming zooplankton from the upper portion of the sample vial. During cold winter months this separation was not necessary due to the absence of appreciable phytoplankton. Microscope slides were prepared using one drop of the zooplankton sample. This method of counting plankton is described in the IBP Handbook, Number 12 (1971).

The remainder of the zooplankton was collected on 7  $\mu$  mesh bloting cloth and rinsed into glass counting vials with ethanol. The samples were counted to determine  $^{134}\text{Cs}$  and  $^{65}\text{Zn}$  activities, dried and weighed. The dried zooplankton was then wet ashed in nitric acid under pressure (Adrian, 1971) and the resultant solution subjected to atomic absorption analysis.

#### Fish

Fish samples were collected in traps constructed with concave funnels on both ends of an aluminum cylinder. These traps were not baited unless necessary and this was only during the mid-winter months when fish were inactive.

The fish were weighed, measured and sexed. The stomach and intestinal contents were removed and stored in 10 percent formaldehyde, counted and separated into benthic, planktonic and plant portions to be dried and weighed. The fish, without gut contents, were dissolved in nitric acid. After distilling the excess nitric acid from the sample, the residue was rinsed into counting vials and the gamma activities determined. The residue was then rinsed into a polyethylene bottle, distilled water was added to dilute the acid to 1.0 N and this solution was tightly capped and heated in a boiling water bath until the residue was completely dissolved. This solution was analyzed for total Cs and Zn.

#### Sediment

Sediment samples were collected using a 1 cm inside diameter, plexiglas coring device. The top 2 cm of each core, containing 100 percent of the radioisotopes in the sediment, as will be shown later, was analyzed routinely. These cores were counted to determine gamma activities, dried and weighed. The stable and radioisotopes were extracted from the sediment using nitric acid and refluxing under pressure in tightly capped vials. The sediment was removed from the acid by centrifugation and thoroughly

rinsed with distilled water, removing all detectable  $^{134}\mathrm{Cs}$  and  $^{65}\mathrm{Zn}$  activity. This solution was analyzed for total Cs and Zn concentrations using atomic absorption techniques.

#### Primary Producers

Grab samples of macrophytes, including roots, were taken from random locations in the ponds and counted to determine gamma activities. Wet ashing in nitric and perchloric acid dissolved the plant tissue for atomic absorption analysis. The same techniques were used for the filamentous periphyton component.

Standing crops of primary producers were estimated only at the end of the study period as the sampling techniques used to mak? these estimates destroyed the integrity of the pond system by mixing sediments and removing large masses of material. Macrophytes were quantitatively samples by placing a metal cylinder over the macrophytes and removing all the plants in the cylinder. Periphyton samples were collected from random locations on the walls of the pond with a hard rubber window squeege, equipped with a 20  $\mu$  mesh bag to collect the material. These samples were scraped from the bottom to the water's surface to negate the effect of depth distribution of the periphyton. Dry weights were determined for the primary producers and the standing crops in each of the four ponds were calculated.

#### RESULTS AND DISCUSSION

## Effects of Isotope Addition

Natural concentrations of \$134\text{Cs}\$ and total Cs in fresh water are extremely low, fractional pCi/kg and ng/kg respectively (Seelye, 1971). The concentrations of \$65\text{Zn}\$ in fresh water are also extremely low, below detectable limits (0.5 pCi/g) for the methods used in this study. In order to insure precision in measuring these isotopes, with a minimum number of preconcentration steps, small amounts of these isotopes were added to the ponds. A small amount (7.134 g) of stable Zn was also added to the ponds. This amount of Zn was determined to be only about 1.5 percent of the Zn that was in the pond system naturally. Thus, the addition of stable Zn was not expected to affect the biota of the treated ponds.

Once dispursed throughout the system the amount of radioisotope added to each pond (6.0 mCi) was below levels which produce chronic effects in aquatic organisms (Auerbach, 1961). The addition of stable Cs to the ponds also had no effect on the biota. This element is categorized as a scarcely toxic element by Bowen (1966). Replacement of K by Cs is the main mode of action of Cs. Because K is an electrochemically important element and Cs does not function similarly when it replaces K, toxic effects are possible.

Concentrations of K in living tissue range between 3000 to 15,000 mg/kg (Bowen, 1966). The amount of Cs necessary to replace sufficient K to cause any toxic effects is far in excess of the amount added to these pond systems.

Zooplankton are very sensitive to high concentrations of metals, especially Zn (Hutchinson, 1957). Data describing seasonal trends in zooplankton abundances and standing crops should reflect any effect of spiking the ponds. Zooplankton data for all four ponds are given in Figure 1. The seasonal trends in zooplankton standing crops do not show any obvious differences due to the addition of isotopes. Numbers of zooplankton per kg of water, for the major groups, are given in Figures 2 through 5. These data suggest that the isotope additions had no observable effect on these four groups of zooplankton. The seasonal trends in these data agree with data presented by McIntosh (1972), collected from similar ponds at the same research facility during 1971.

All four ponds showed similar seasonal trends for gross primary productivities (Figure 6). The values of gross primary productivity calculated in this study agree with data presented by Haines (1971).

## Cs and Zn Isotope Distributions

<u>Water</u> - The Cs and Zn isotope concentrations and specific activities for water are given in Figures 7 through 12.

One requirement necessary to insure the validity of the specific activity hypothesis, is that the system must be in equilibrium with respect to the isotope distribution. Figure 1. Changes in zooplankton standing crops over time. Each point represents the mean of five samples. The bars represent plus or minus one standard error of the mean. Standard errors were smaller than the points where only points are shown Vertical striped bars indicate the addition of isotopes at that time. These data are on a dry weight basis.

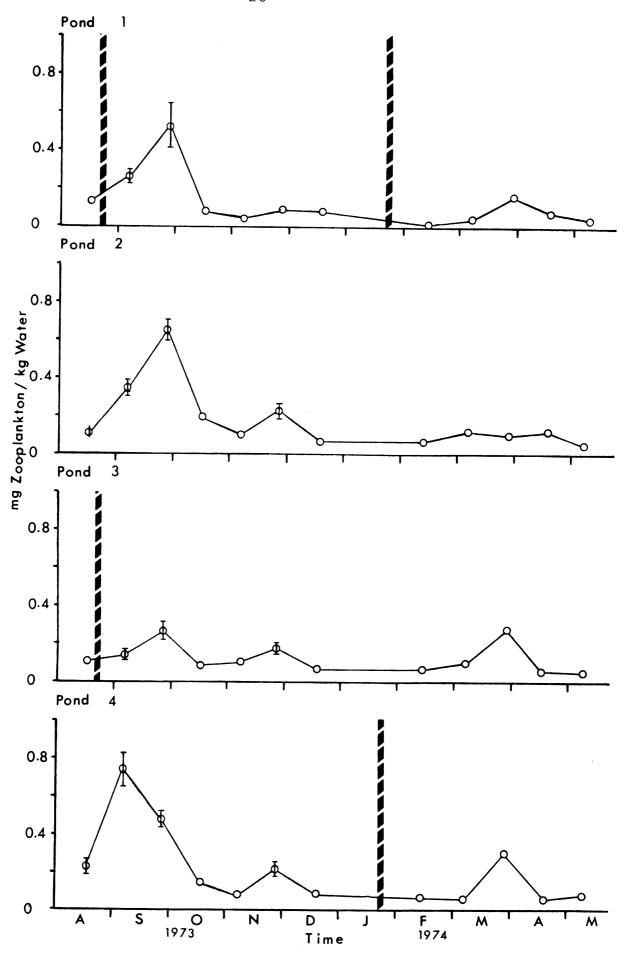


Figure 2. Changes in Pond 1 zooplankton numbers over time. Each point represents the mean of five samples. The bars represent plus or minus one standard error of the mean. Standard errors were smaller than the points where only points are shown.

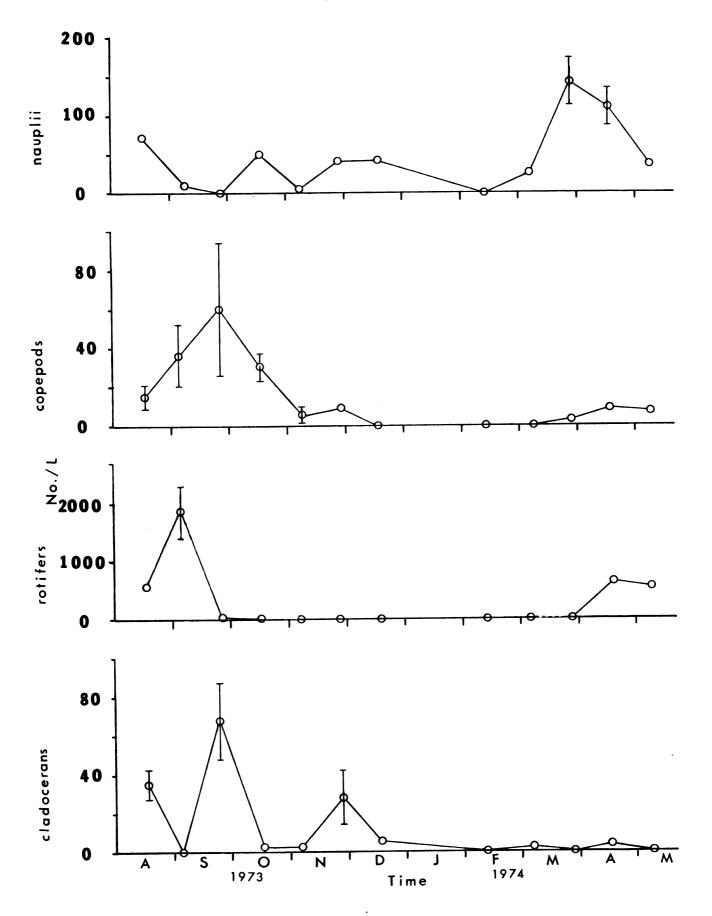


Figure 3. Changes in Pond 2 zooplankton numbers over time. Each point represents the mean of five samples. The bars represent plus or minus one standard error of the mean.

Standard errors were smaller than the points where only points are shown.

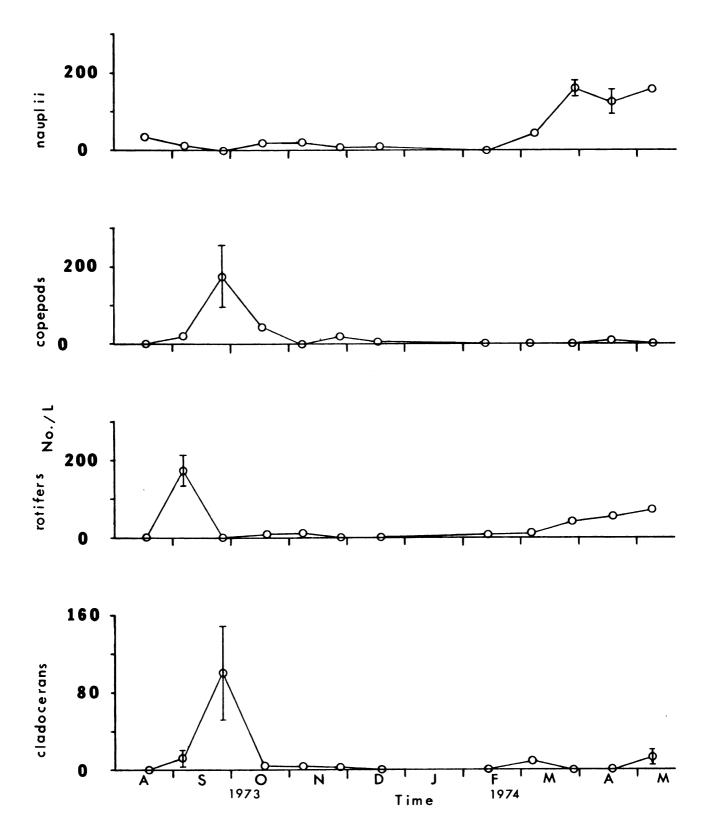


Figure 4. Changes in Pond 3 zooplankton numbers over time. Each point represents the mean of five samples. The bars represent plus or minus one standard error of the mean.

Standard errors were smaller than the points where only points are shown.

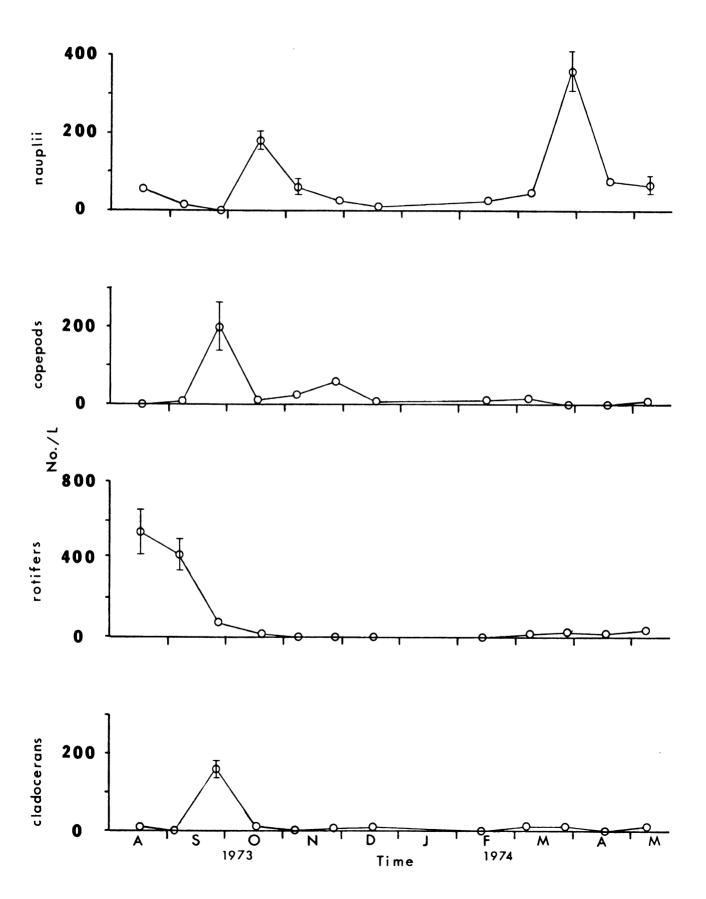


Figure 5. Changes in Pond 4 zooplankton numbers over time. Each point represents the mean of five samples. The bars represent plus or minus one standard error of the mean. Standard errors were smaller than the points where only points are shown.

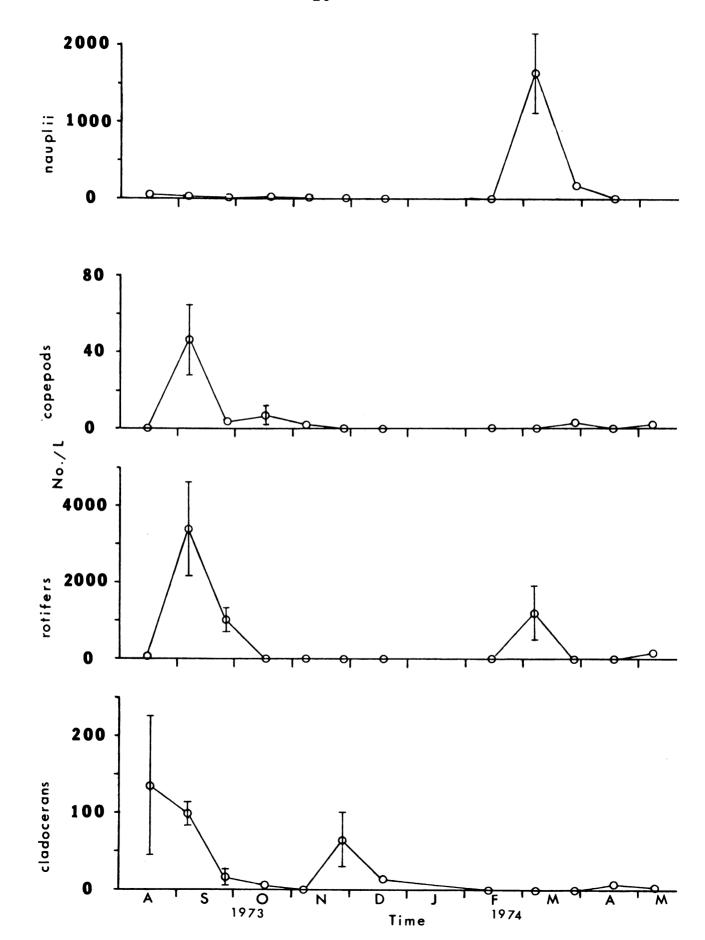


Figure 6. Changes in gross primary productivities (GPP) over time. Points represent one value based on diurnal oxygen.

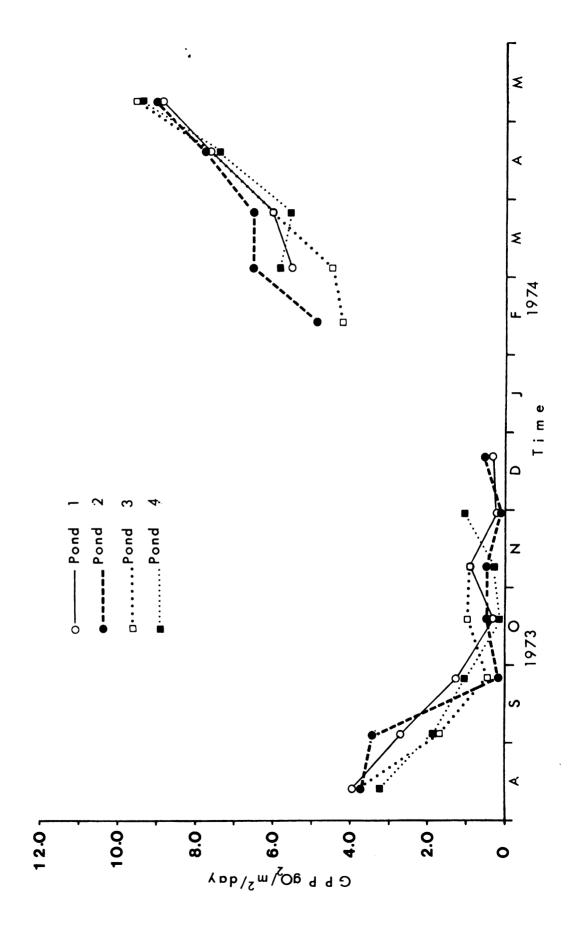


Figure 7. Changes in Cs isotope concentrations and specific activities (S.A.) over time in Pond 1 water. Each point represents the mean of five samples. The bars represent plus or minus one standard error of the mean. Standard errors were smaller than the points where only points are shown. Solid points indicate calculated concentrations and specific activity at the time of spiking. Vertical striped bars represent isotope additions.

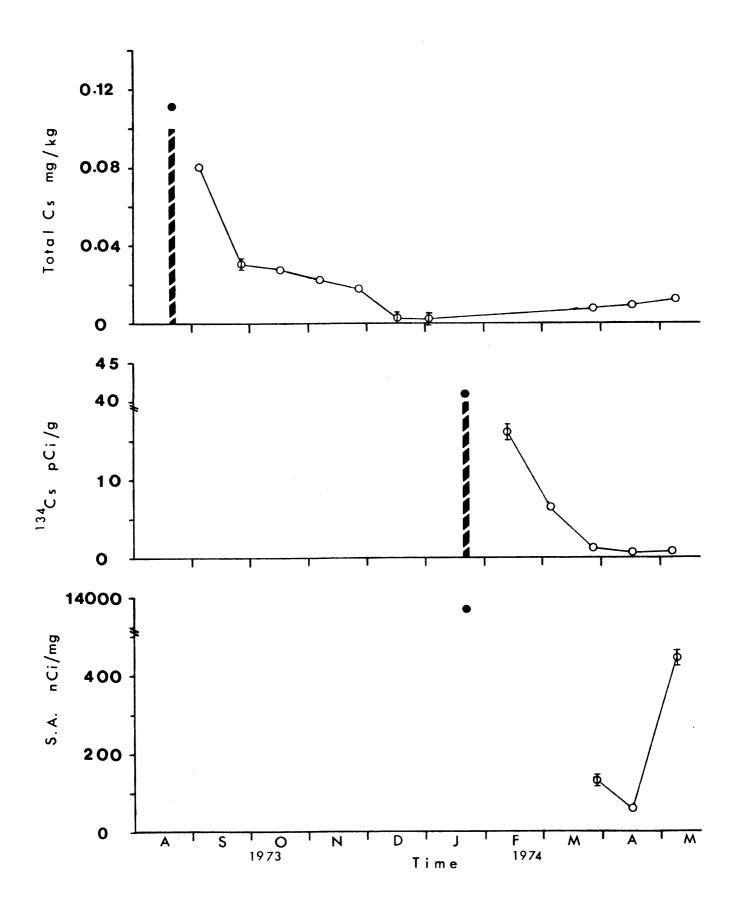


Figure 8. Changes in Zn isotope concentrations and specific activities (S. A.) over time in Pond 1 water. Each point represents the mean of five samples. The bars represent plus or minus one standard error of the mean. Standard errors were smaller than the points where only points are shown. Solid points indicate calculated concentrations and specific activity at the time of spiking. Vertical striped bars represent isotope additions.

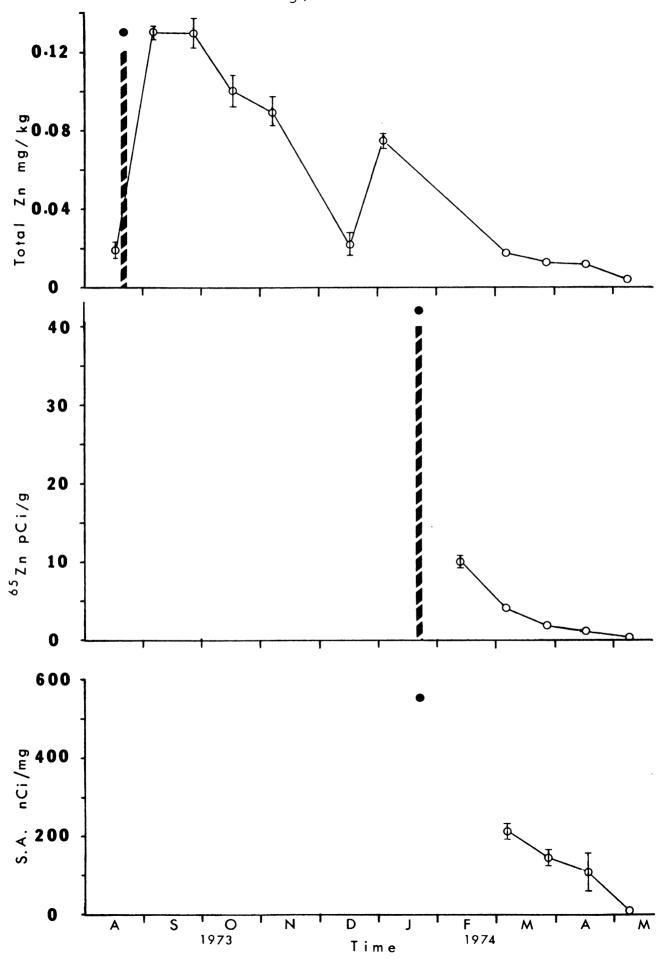


Figure 9. Changes in Cs isotope concentrations and specific activities (S. A.) over time in Pond 3 water. Each point represents the mean of five samples. The bars represent plus or minus one standard error of the mean. Standard errors were smaller than the points where only points are shown. Solid points indicate calculated concentrations and specific activity at the time of spiking. Vertical striped bars represent isotope additions.

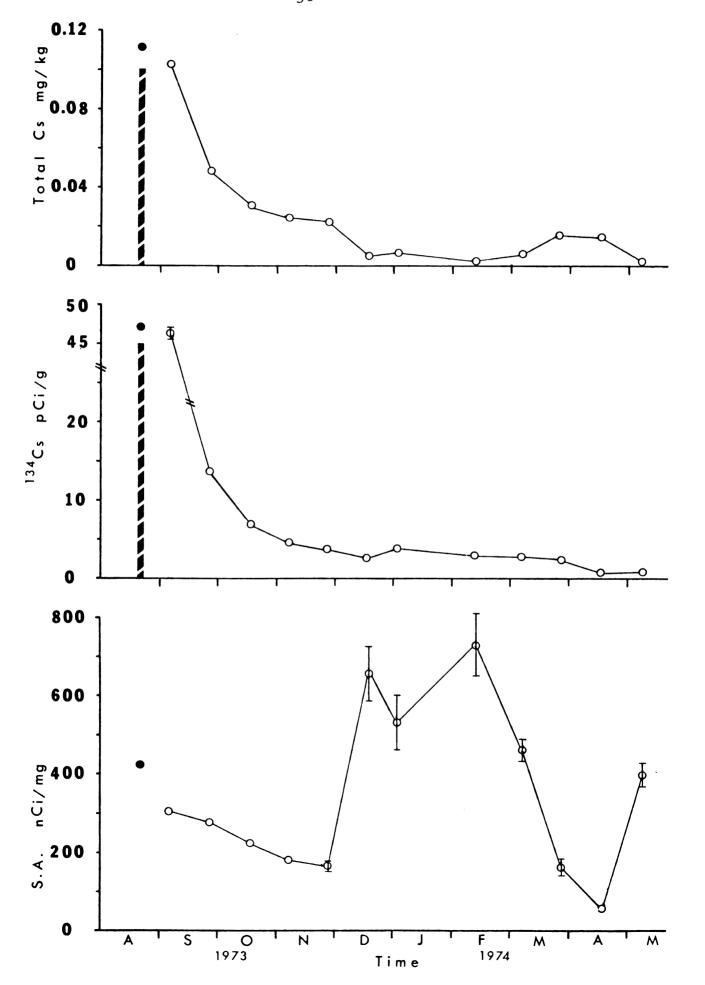


Figure 9. Changes in Cs isotope concentrations and specific activities (S. A.) over time in Pond 3 water. Each point represents the mean of five samples. The bars represent plus or minus one standard error of the mean. Standard errors were smaller than the points where only points are shown. Solid points indicate calculated concentrations and specific activity at the time of spiking. Vertical striped bars represent isotope additions.

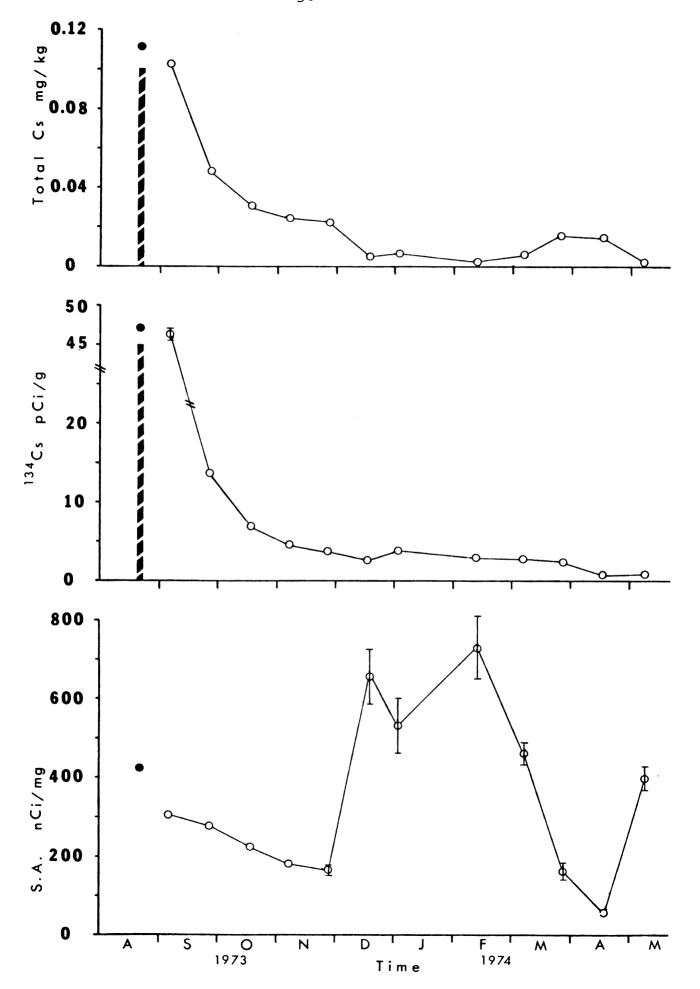


Figure 10. Changes in Zn isotope concentrations and specific activities (S. A.) over time in Pond 3 water. Each point represents the mean of five samples. The bars represent plus or minus one standard error of the mean. Standard errors were smaller than the points where only points are shown. Solid points indicate calculated concentrations and specific activity at the time of spiking. Vertical striped bars represent isotope additions.

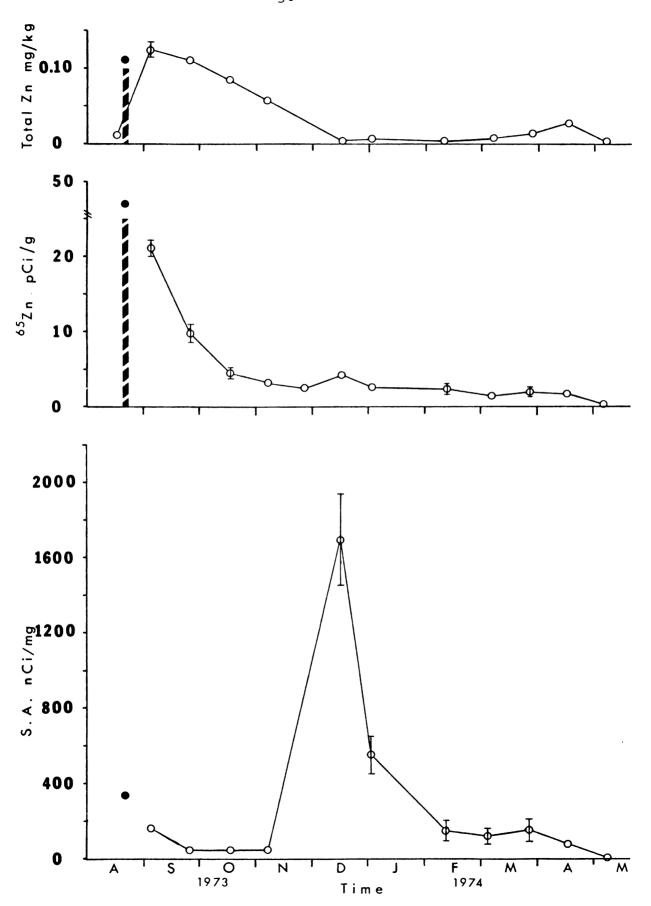


Figure 11. Changes in Cs isotope concentrations and specific activities (S. A.) over time in Pond 4 water. Each point represents the mean of five samples. The bars represent plus or minus one standard error of the mean. Standard errors were smaller than the points where only points are shown. Solid points indicate calculated concentrations and specific activity at the time of spiking. Vertical striped bars represent isotope additions.

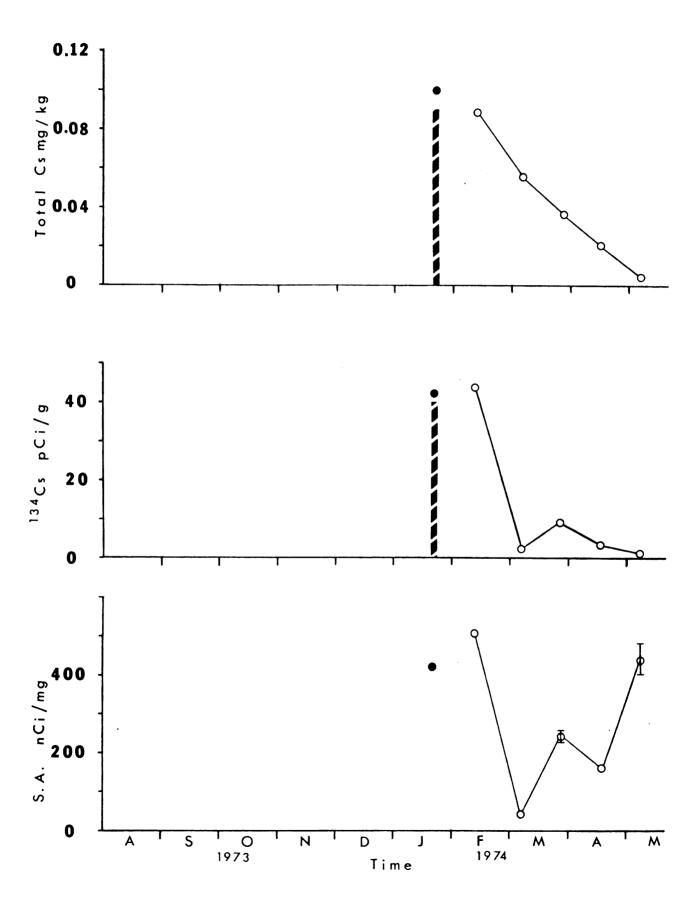
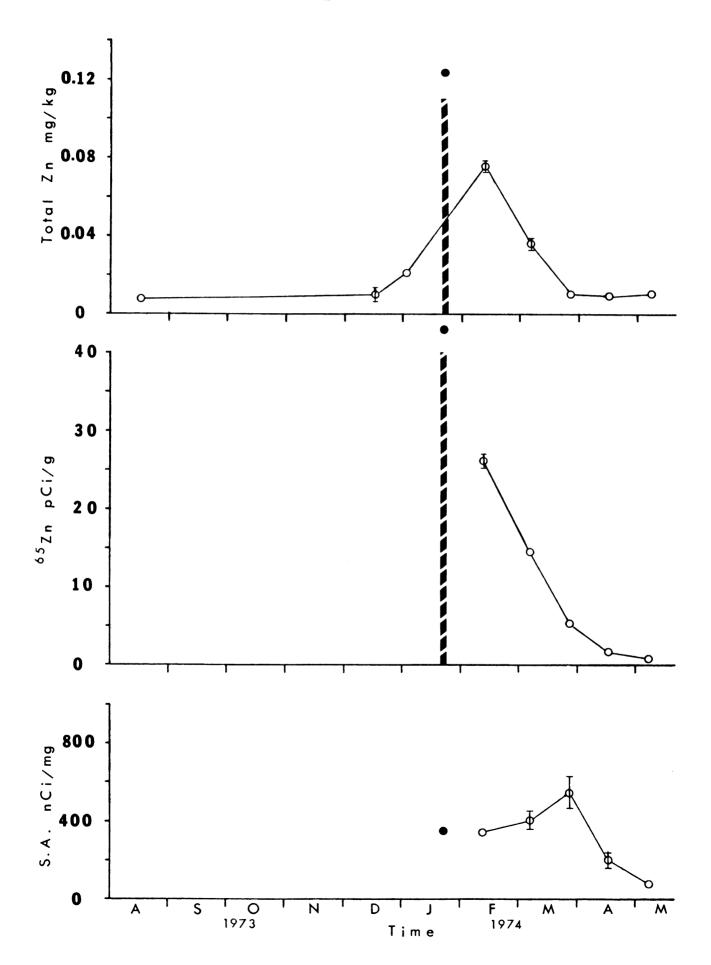


Figure 12. Changes in Zn isotope concentrations and specific activities (S. A.) over time in Pond 4 water. Each point represents the mean of five samples. The bars represent plus or minus one standard error of the mean. Standard errors were smaller than the points where only points are shown. Solid points indicate calculated concentrations and specific activity at the time of spiking. Vertical striped bars represent isotope additions.



The Cs and Zn isotope concentrations have reached a plateau by mid-study. This can be interpreted as an equilibrium condition (Figures 9 and 10). The following discussion of trends in isotope concentrations and specific activities will be mainly concerned with data from Pond 3 because these data represent the entire 10 months of the study (a function of the spiking schedule). Trends in isotope concentrations and specific activities are similar in the other ponds, in general, but data are available for all parameters for only the last half of the study period.

Data presented in Figure 10 illustrate the decrease in Zn isotope concentrations in Pond 3 water. The rate of decrease in total Zn concentrations appears to be less than the rate of decrease of  $^{65}$ Zn concentrations. However, this is an artifact of the units on the data presented. The rate of total Zn concentration decrease cannot be compared directly to the rate of radioisotope concentration decrease. Total Zn units are on a mass basis while  $^{65}$ Zn units are radioactivity measurements. The linear decrease in total Zn concentration suggests that some of the Zn present prior to spiking is being released to the water from the other components. The curvalinear relationship of the 65Zn concentrations indicates that the rate of <sup>65</sup>Zn removal from the water, changes over time. Initially all the  $^{65}$ Zn in the system was in the water. As the water  $^{65}$ Zn concentration approached equilibrium the rate of removal from thewater decreased. This was probably due to the release of  $^{65}$ Zn by the other components.

The data presented in Figure 9 for Cs demonstrates the same relationship. However, the difference in the shapes of the curves between total Cs and <sup>134</sup>Cs is not well defined. This suggests that if a pool of stable Cs existed before spiking, it was small relative to the Zn pool.

The presence of existing pools of stable Cs and Zn before spiking and the magnitude of these pools are important factors affecting the specific activities in all components of the system, as will be discussed later.

The points marked "calculated concentrations" or "cal-culated specific activity," on the figures representing water data, are values claculated using the total volume of water in the ponds and the amount of each isotope added, including the concentration of stable Zn present prior to spiking.

The <sup>134</sup>Cs specific activities, in the water of Pond 3, are shown in Figure 9. These data show the large amount of variation in specific activities over time, ranging from 50 to 725 nCi/mg and was lower than the calculated specific activity over time is due to the exchange of the stable Cs, present in the system prior to the spike, between the components. Identification of the details of the rates of exchange and the components responsible for the changes in the water specific activity is not possible from the data collected in this study. If no stable Cs had been present in the system, prior to spiking, the calculated <sup>134</sup>Cs specific activity would prevail throughout the system, assuming no difference in physiochemical behavior of the Cs isotopes. Because some stable Cs appears to have been present, the initial

water specific activity is lower than the calculated specific activity. However, by the end of the study, the observed water  $^{134}\text{Cs}$  specific activity is close to the calculated specific activity.

The <sup>65</sup>Zn specific activity, Figure 10, at the beginning of the study, is considerably less than the calculated specific activity. This is due again, to the large pool of stable Zn in the system. The change in <sup>65</sup>Zn specific activity over time is probably due to the exchange of Zn isotopes between components and cannot be adequately explained by these data. The final (May) specific activity was 13.28 nCi/mg and was lower than the calculated specific activity (380 nCi/mg). This difference is due to the presence of stable Zn prior to the addition of the isotopes to the ponds.

## Zooplankton

Concentrations of the isotopes of Cs and Zn and specific activities, over time, are shown in Figures 13 through 18, for the zooplankton.

Changes in Cs isotope concentrations in zooplankton over time were similar to those of the water in corresponding ponds. Considering Pond 3 (Figure 15) in detail, it appears that after approximately four months, a leveling of the Cs isotope concentrations has occurred. This suggests that a rapid equilibrium was achieved in this low trophic level. The Zn concentrations fluctuate throughout the year in Pond 3, as can be seen in Figure 16. There appears to be two peak Zn isotope concentrations, one in

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Figure 13. Changes in Cs isotope concentrations and specific activities (S. A.) over time in Pond 1 zooplankton. Each point represents the mean of five samples. The bars represent plus or minus one standard error of the mean. Standard errors were smaller than the points where only points are shown. Vertical striped bars represent isotope additions. These data are based on dry weights.

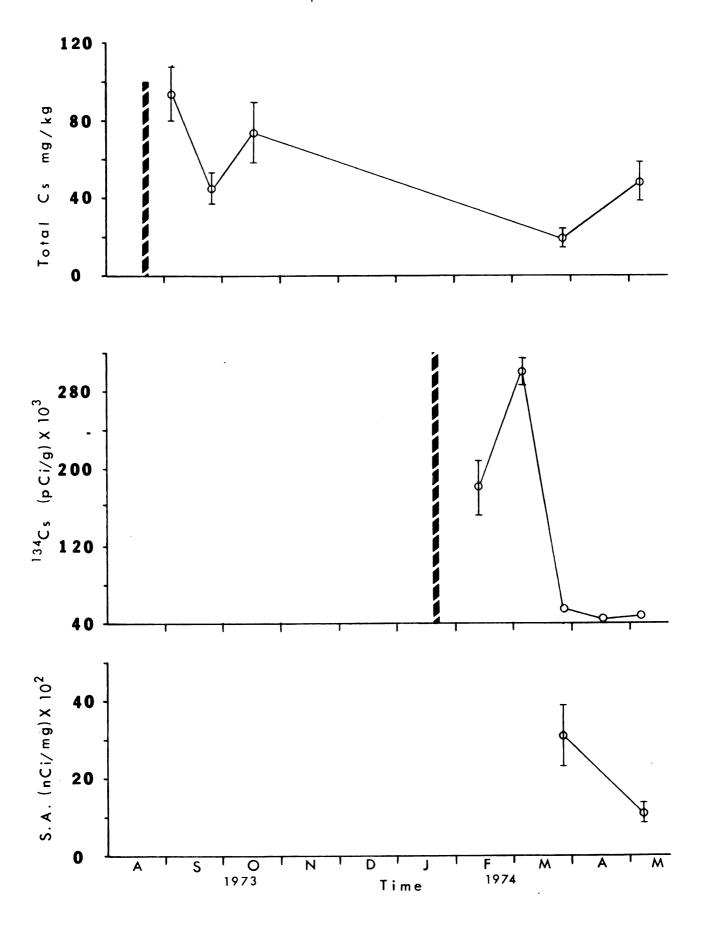
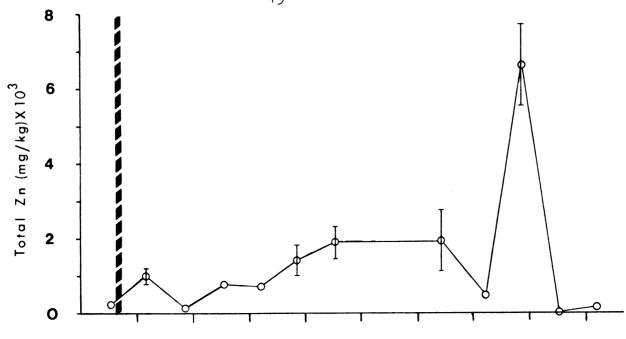
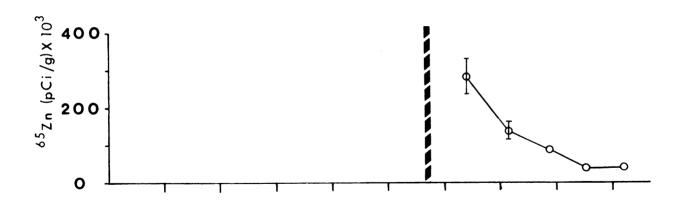


Figure 14. Changes in Zn isotope concentrations and specific activities (S. A.) over time in Pond 1 zooplankton. Each point represents the mean of five samples. The bars represent plus or minus one standard error of the mean. Standard errors were smaller than the points where only points are shown. Vertical striped bars represent isotope additions. These data are based on dry weights.





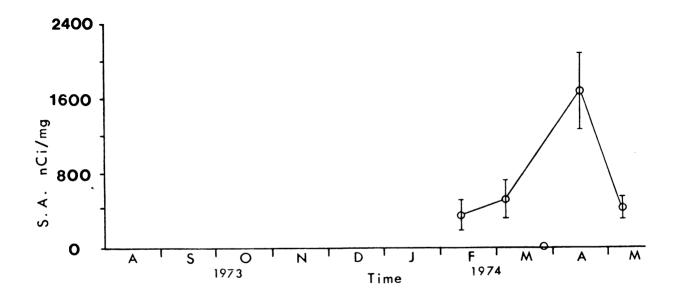
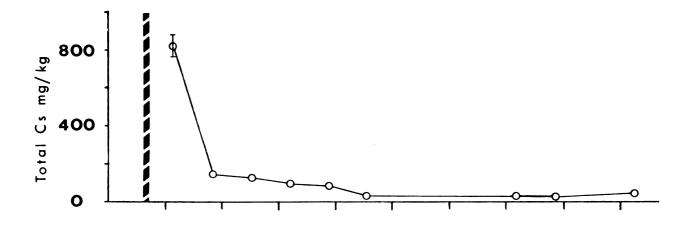
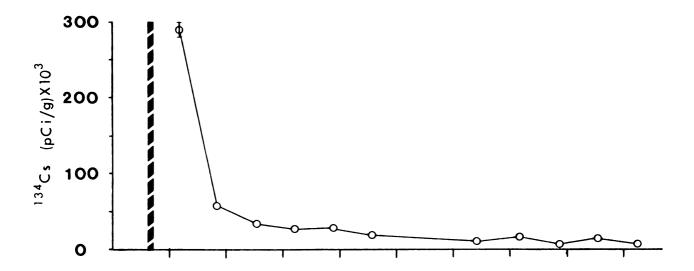


Figure 15. Changes in Cs isotope concentrations and specific activities (S. A.) over time in Pond 3 zooplankton. Each point represents the mean of five samples. The bars represent plus or minus one standard error of the mean. Standard errors were smaller than the points where only points are shown. Vertical striped bars represent isotope additions. These data are based on dry weights.





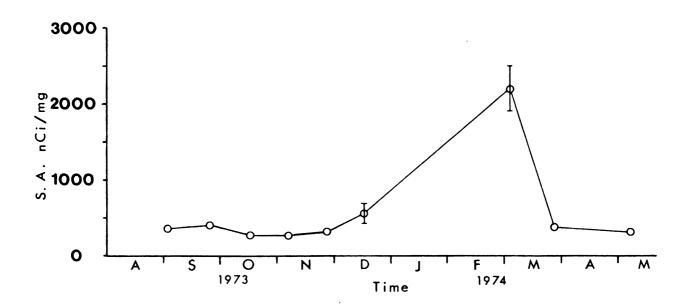


Figure 16. Changes in Zn isotope concentrations and specific activities (S. A.) over time in Pond 3 zooplankton. Each point represents the mean of five samples. The bars represent plus or minus one standard error of the mean. Standard errors were smaller than the points where only points are shown. Vertical striped bars represent isotope additions. These data are based on dry weights.

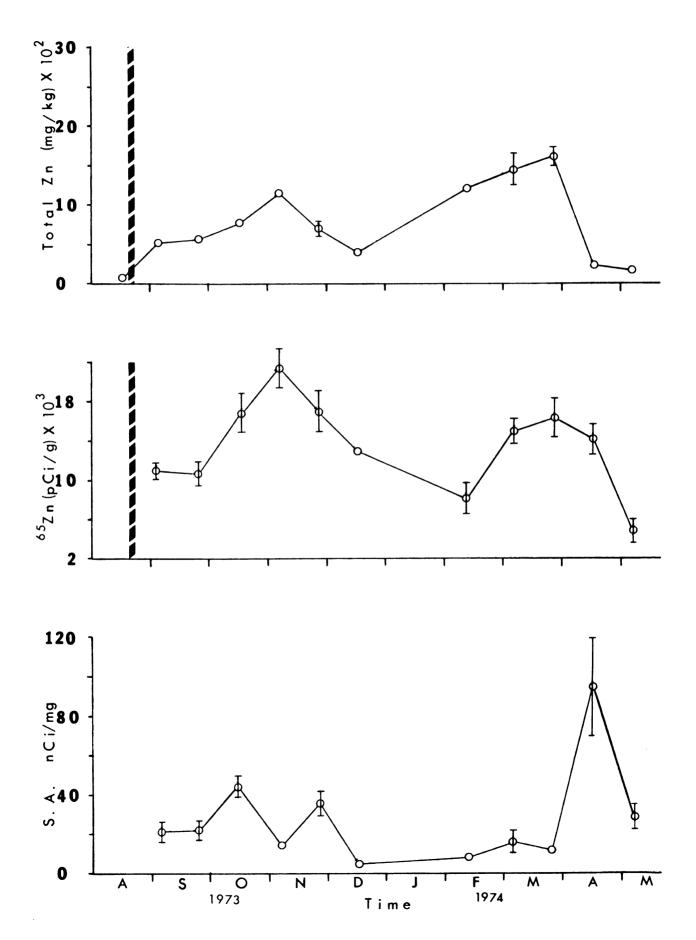


Figure 17. Changes in Cs isotope concentrations and specific activities (S. A.) over time in Pond 4 zooplankton. Each point represents the mean of five samples. The bars represent plus or minus one standard error of the mean. Standard errors were smaller than the points where only points are shown. Vertical striped bars represent isotope additions. These data are based on dry weights.

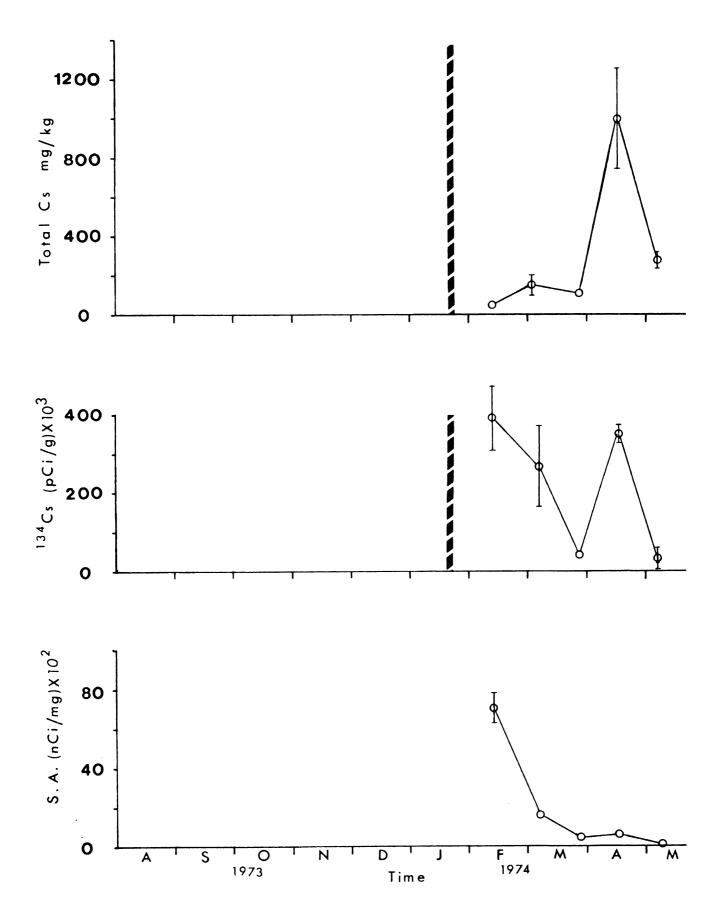
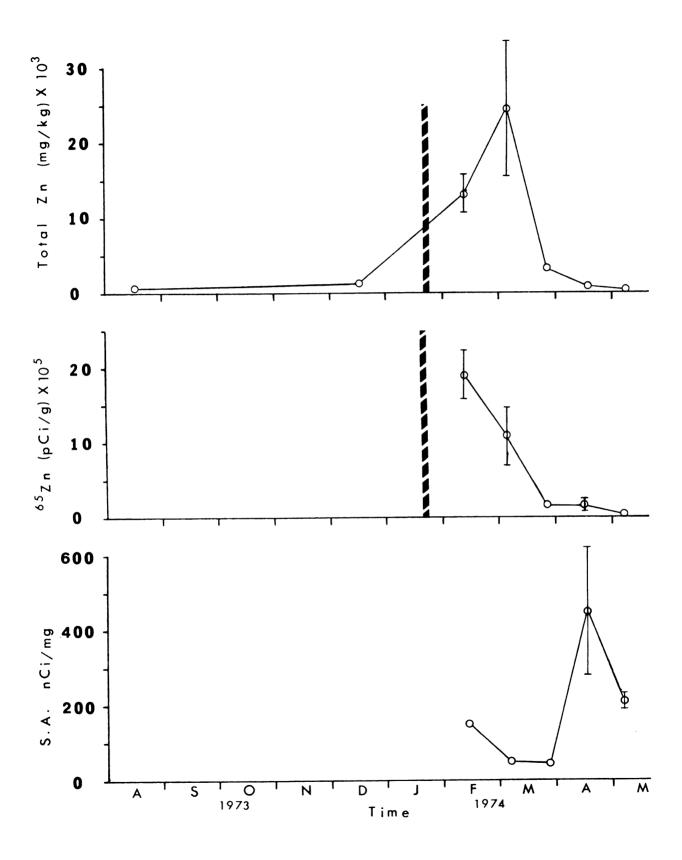


Figure 18. Changes in Zn isotope concentrations and specific activities (S. A.) over time in Pond 4 zooplankton. Each point represents the mean of five samples. The bars represent plus or minus one standard error of the mean. Standard errors were smaller than the points where only points are shown. Vertical striped bars represent isotope additions. These data are based on dry weights.



late fall (October to November) and one in the spring (March). These peaks compare favorably with the peaks in secondary production, as shown in Figure 1, with a lag time of about one month between peak standing crop and peak concentrations. The numbers per liter of the four major groups of zooplankton, Figure 4, in Pond 3, indicate that the peaks in nauplii correspond to the peaks in zooplankton standing crops. As the nauplii mature, the adult forms are more susceptible to predation by fish and thus, the standing crop decreases. At the same time, the adult zooplankton, being more efficient predators (Brooks and Dodson, 1965), consume more food and concentrate more isotope.

The <sup>134</sup>Cs specific activities of the zooplankton in Pond 3, shown in Figure 15, show trends similar to the trends shown for the water from this pond (Figure 9).

The variability of the <sup>65</sup>Zn specific activities is shown in Figure 16. This variability is similar to that shown for the water specific activities (Figure 10). The stable Zn present in the ponds, prespike, was in equilibrium. This is shown in the data for Pond 2 (control pond) (Table 5). The changes in concentrations of Zn in the components of Pond 2 are small throughout the study period. The partitioning of Zn in the components of Pond 2 remained stable throughout the study period, as will be shown later. The <sup>65</sup>Zn should pass between the components at the same rate as the stable Zn but the specific activities may vary due to the stable Zn already present in the components in varying concentrations. The final <sup>65</sup>Zn specific activity for zooplankton was higher than the final water specific activity,

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possibly because the  $^{65}$ Zn was retained in this component longer than in the water. Zooplankton require Zn for metabolism and would therefore retain this element (Bowne, 1966).

## Fish

The Cs and Zn isotope concentrations for Ponds 1, 3 and 4 are given in Figures 19 through 24. The specific activities are also given in these figures.

After four months, from the time of isotope addition, in Ponds 1 and 3, both Cs and Zn isotope concentrations seem to have reached a plateau. Some increase in concentration takes place in the spring, probably due to increased feeding, shown in Figure 25, for Pond 3.

An attempt was made to determine whether a definable relationship existed between  $^{134}\text{Cs}$  and  $^{65}\text{Zn}$  activities and feeding habits of the fish. A series of plots, showing food selection by fish, is given in Figure 26. Examination of these data failed to suggest any relationship between feeding habits and radioisotope concentrations in the gut contents.

This discussion of specific activity again, centers on Pond 3. The <sup>134</sup>Cs specific activities were consistant over time, as can be seen in Figure 21. Unfortunately benthos samples were not analyzed for isotope concentrations because of problems in disturbing the sediment to the degree necessary to collect sufficiently large macroinvertebrate samples. Figure 26 shows a peak in feeding on benthic material in March. At this time and after, the specific activities,

Table 5. Isotope concentrations in water, zooplankton and fish from Pond 2. Means of five samples  $\pm$  1 S. E.

	Total Cs mg/kg	13 <sup>4</sup> Cs pCi/g	Total Zn mg/kg	65 <sub>Zn</sub> nCi/g
Water				
Pre-spike	B.D.*	B.D.	0.004±0.0006	B.D.
Mid-study	B.D.	B.D.	0.015±0.0030	B.D.
End-study	В.Д.	B.D.	0.005±0.0007	B.D.
Zooplankton				
Pre-spike	B.D.	B.D.	162.2±37.89	B.D.
Mid-study	B.D.	B.D.	287.8±30.39	B.D.
End-study	В.Д.	B.D.	147.7±39.14	B.D.
Fish				
Pre-spike	B.D.	B.D.	32.52±1.260	B.D.
Mid-study	B.D.	B.D.	31.96±2.660	B.D.
End-study	В.Д.	B.D.	34.54±2.470	B.D.

<sup>\*</sup>Below detectable limits.

Table 5. Isotope concentrations in water, zooplankton and fish from Pond 2. Means of five samples  $\pm$  1 S. E.

	134		65 <sub>Zn</sub>
Total Cs mg/kg	pCi/g	Total Zn mg/kg	nCi/g
B.D.*	B.D.	0.004±0.0006	B.D.
B.D.	B.D.	0.015±0.0030	B.D.
В.Д.	B.D.	0.005±0.0007	B.D.
B.D.	В.Д.	162.2±37.89	B.D.
B.D.	B.D.	287.8±30.39	B.D.
B.D.	B.D.	147.7±39.14	B.D.
B.D.	B.D.	32.52±1.260	B.D.
B.D.	B.D.	31.96±2.660	B.D.
B.D.	B.D.	34.54±2.470	B.D.
	B.D.* B.D. B.D. B.D. B.D. B.D.	mg/kg pCi/g  B.D.* B.D.  B.D. B.D.  B.D. B.D.  B.D. B.D.  B.D. B.D.  B.D. B.D.	B.D.*  B.D. * B.D. 0.004±0.0006  B.D. B.D. 0.015±0.0030  B.D. B.D. 0.005±0.0007  B.D. B.D. 162.2±37.89  B.D. B.D. 287.8±30.39  B.D. B.D. 147.7±39.14  B.D. B.D. 32.52±1.260  B.D. B.D. 31.96±2.660

<sup>\*</sup>Below detectable limits.

Figure 19. Changes in Cs isotope concentrations and specific activities (S. A.) over time in Pond 1 fish. Each point represents the mean of five samples. The bars represent plus or minus one standard error of the mean. Standard errors were smaller than the points where only points are shown. Vertical striped bars represent isotope additions. These data are based on wet weights.

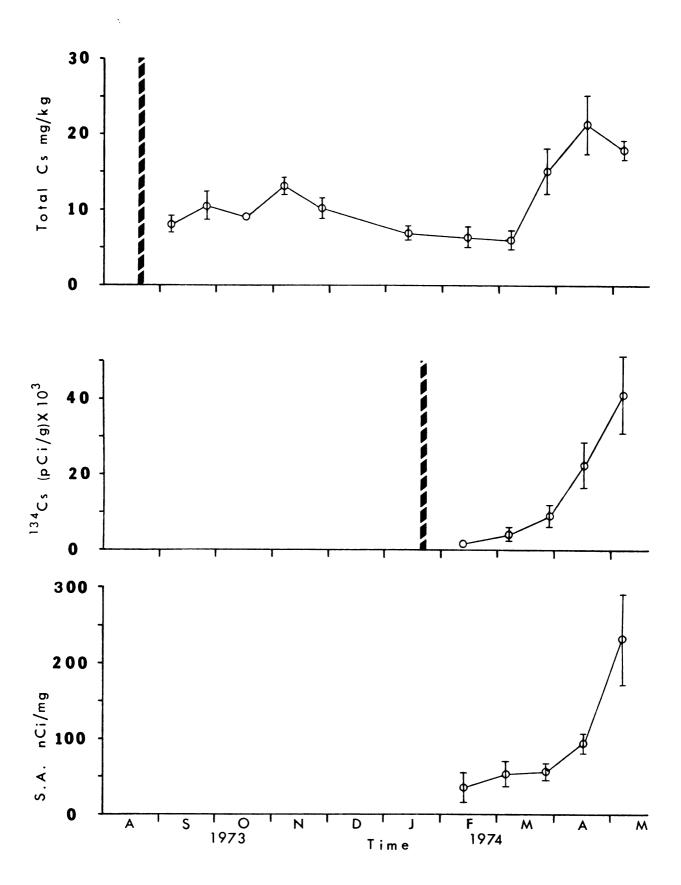


Figure 20. Changes in Zn isotope concentrations and specific activities (S. A.) over time in Pond 1 fish. Each point represents the mean of five samples. The bars represent plus or minus one standard error of the mean. Standard errors were smaller than the points where only points are shown. Vertical striped bars represent isotope additions. These data are based on wet weights.

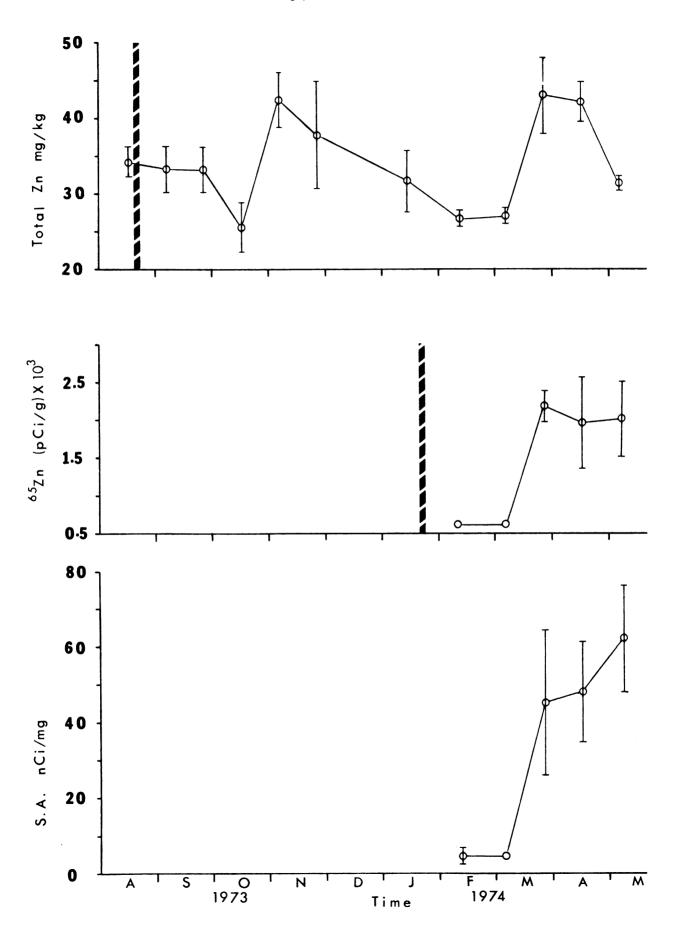


Figure 21. Changes in Cs isotope concentrations and specific activities (S. A.) over time in Pond 3 fish. Each point represents the mean of five samples. The bars represent plus or minus one standard error of the mean. Standard errors were smaller than the points where only points are shown. Vertical striped bars represent isotope additions. These data are based on wet weights.

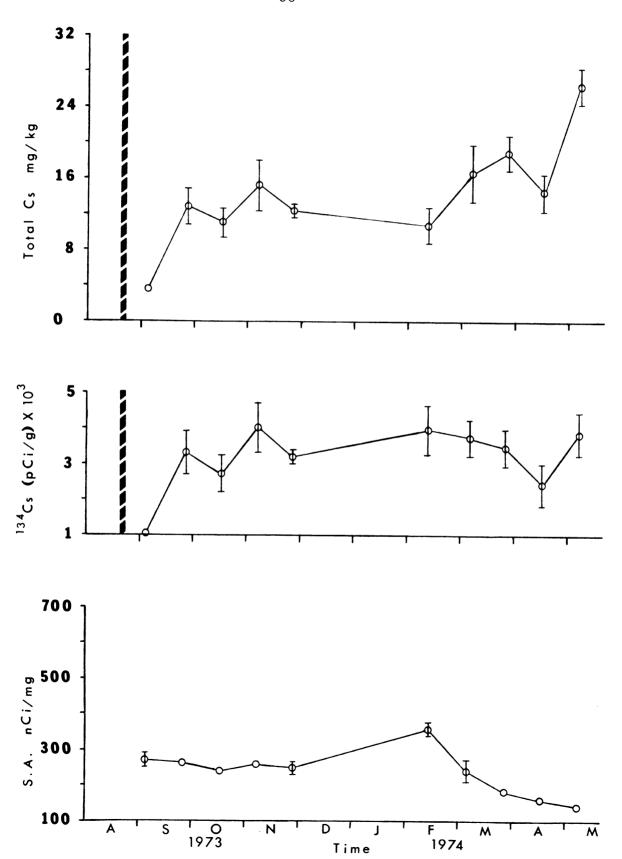
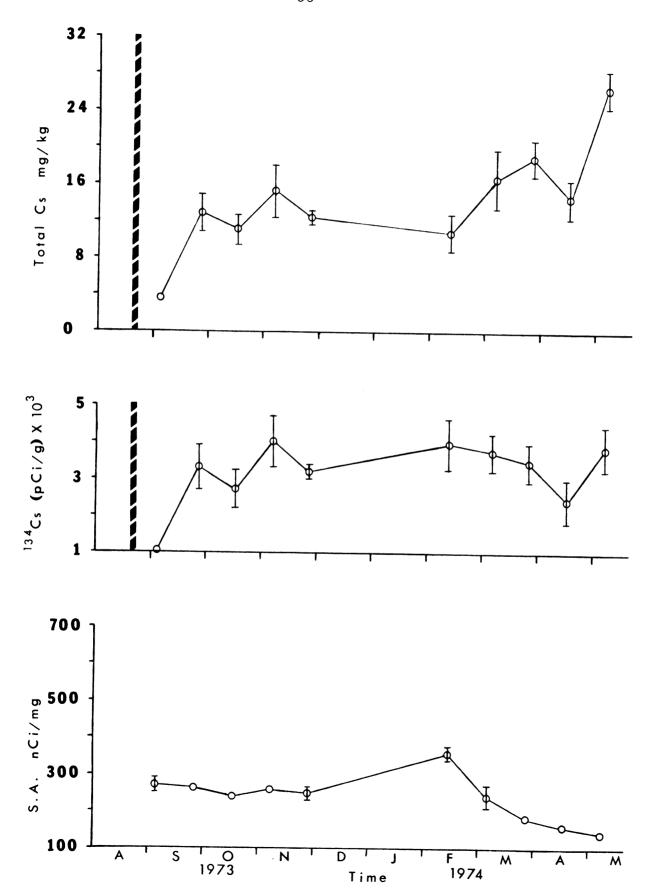


Figure 21. Changes in Cs isotope concentrations and specific activities (S. A.) over time in Pond 3 fish. Each point represents the mean of five samples. The bars represent plus or minus one standard error of the mean. Standard errors were smaller than the points where only points are shown. Vertical striped bars represent isotope additions. These data are based on wet weights.



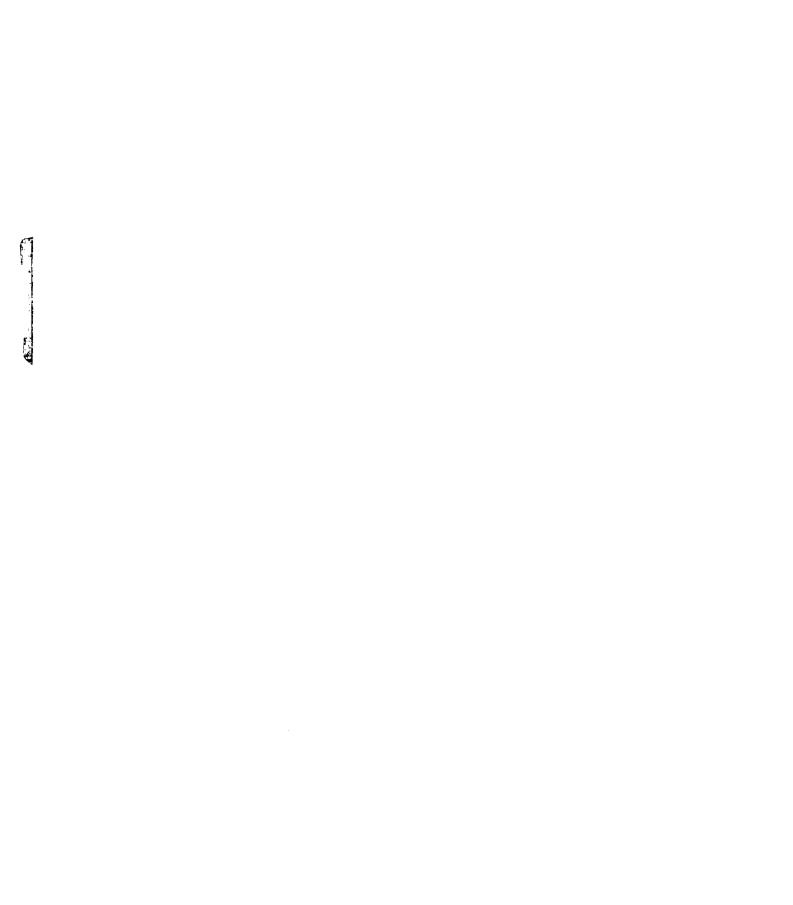
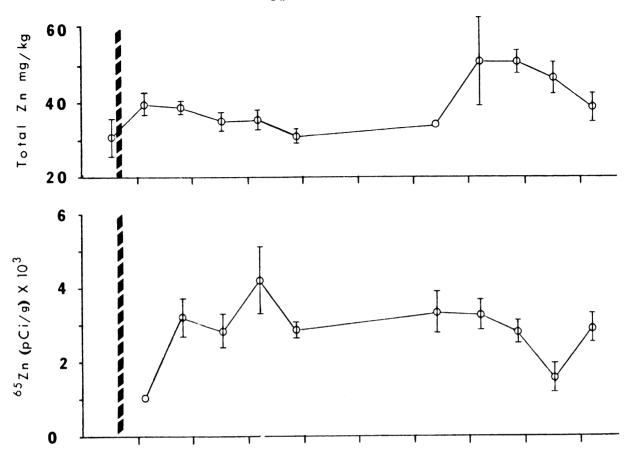


Figure 22. Changes in Zn isotope concentrations and specific activities (S. A.) over time in Pond 3 fish. Each point represents the mean of five samples. The bars represent plus or minus one standard error of the mean. Standard errors were smaller than the points where only points are shown. Vertical striped bars represent isotope additions. These data are based on wet weights.



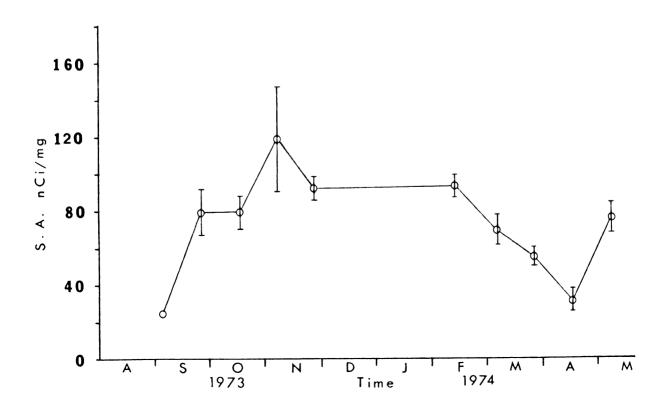


Figure 23. Changes in Cs isotope concentrations and specific activities (S. A.) over time in Pond 4 fish. Each point represents the mean of five samples. The bars represent plus or minus one standard error of the mean. Standard errors were smaller than the points where only points are shown. Vertical striped bars represent isotope additions. These data are based on wet weights.

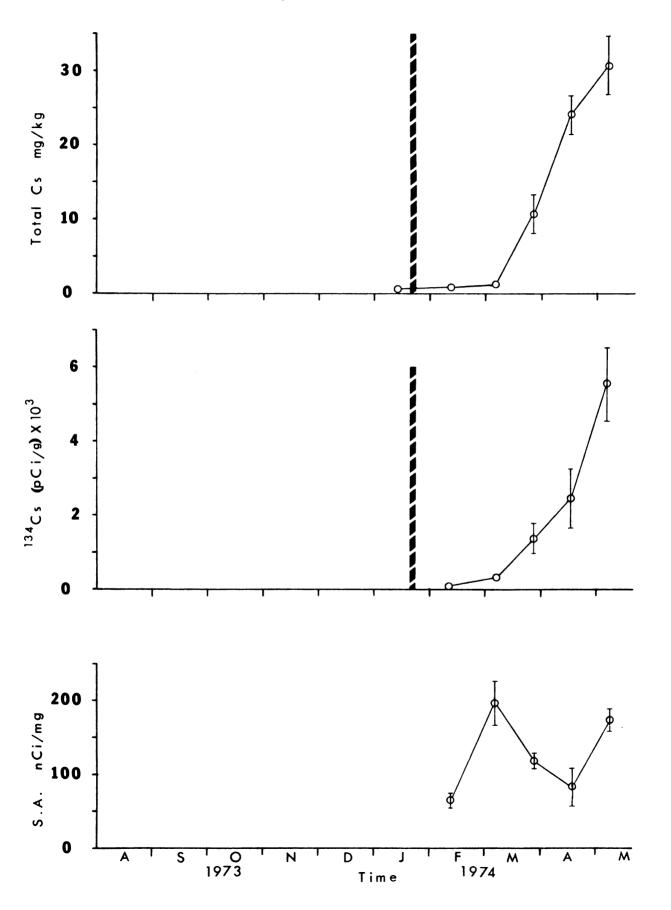


Figure 24. Changes in Zn isotope concentrations and specific activities (S. A.) over time in Pond 4 fish. Each point represents the mean of five samples. The bars represent plus or minus one standard error of the mean. Standard errors were smaller than the points where only points are shown. Vertical striped bars represent isotope additions. These data are based on wet weights.

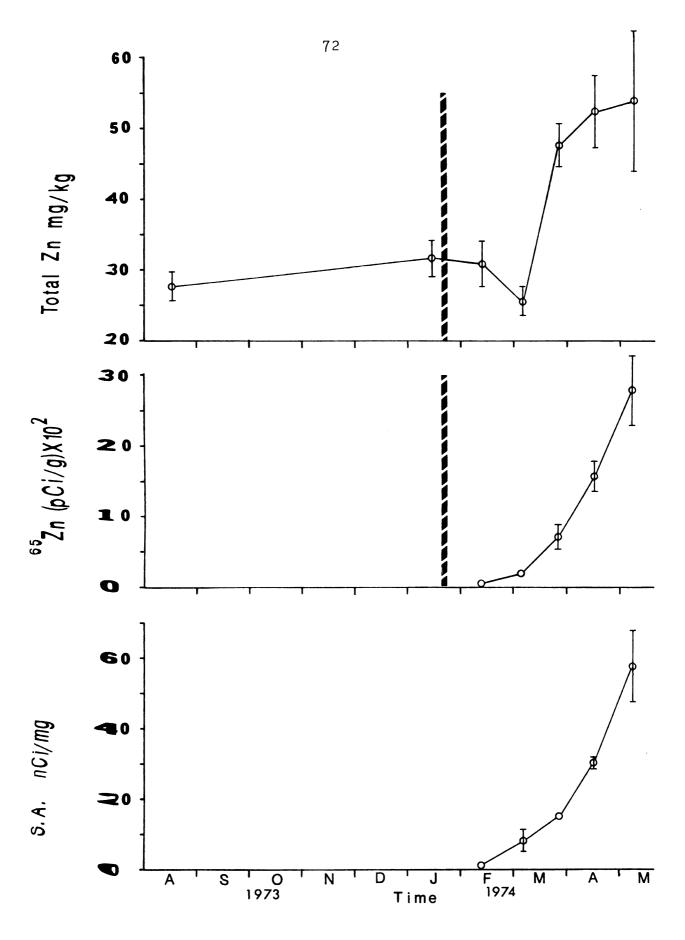


Figure 25. Changes in feeding habits of Pond 3 fish over time. Calculated using means of five samples. Radioisotope concentrations are means of five samples on a dry weight basis.

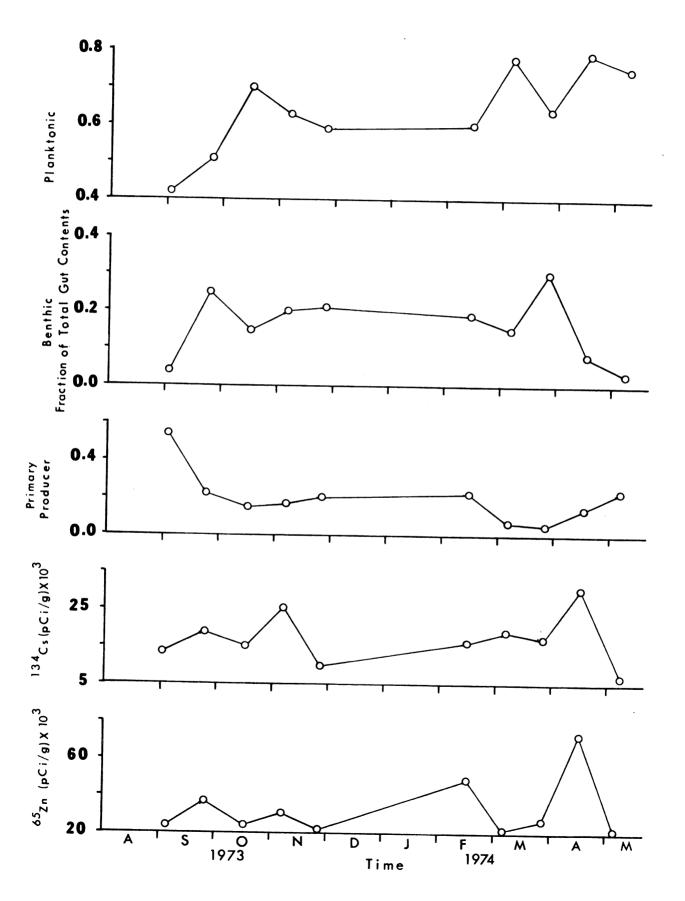
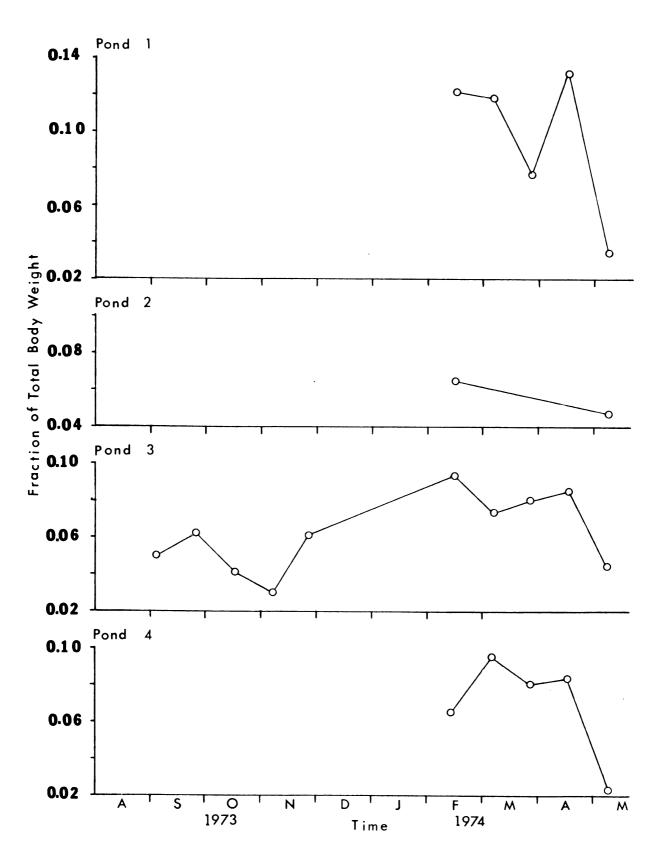
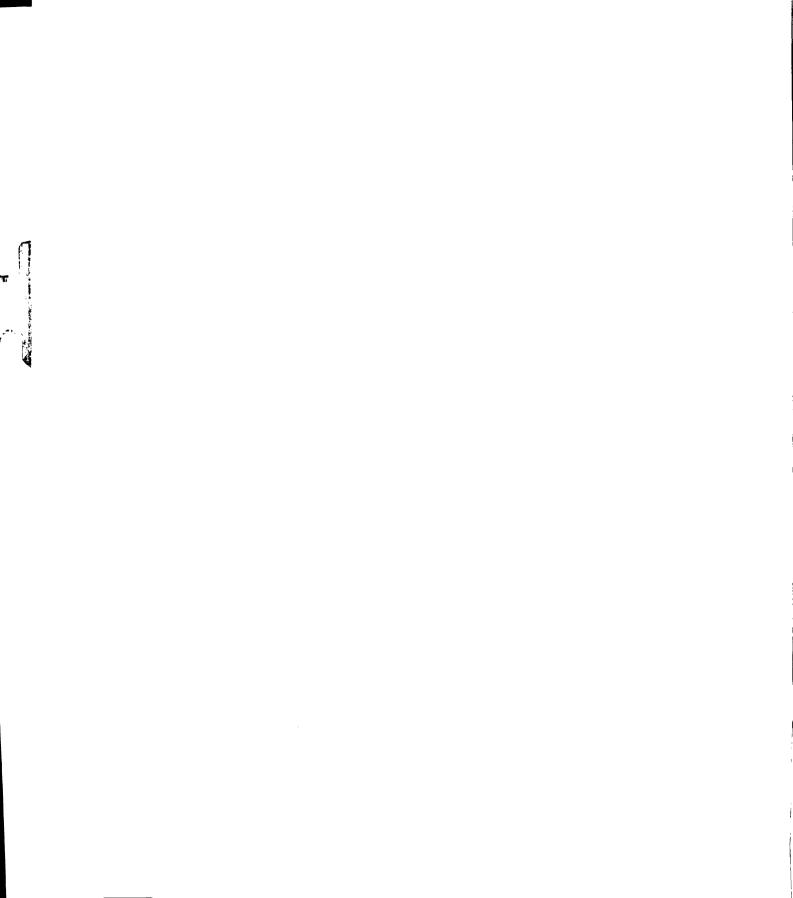


Figure 26. Changes in total gut contents for fish from all four ponds over time. Points were calculated using means of five samples.





in the fish decreased to the end of the study. This might be explained by considering the presence of stable Cs in the sediment before spiking. The presence of more stable Cs in the benthic macroinvertebrates would cause lower specific activities and thus, when the fish were feeding on these materials, their specific activities decreased.

The  $^{65}$ Zn specific activities in the fish were variable over time, as can be seen in Figure 22. The increase in specific activity for the period right after the spike can be attributed to the uptake of the  $^{65}$ Zn while the total Zn concentrations remaining nearly constant. During the winter months, with decreased food consumption, the <sup>65</sup>Zn specific activity remained stable. In the spring, the increased feeding activity and the shift in feeding habits to benthic organisms by the fish, caused an increase in uptake of the stable Zn and a resulting decrease in the  $^{65}$ Zn specific activity. Near the end of the study period the fish show an increase in <sup>65</sup>Zn uptake, possibly because of increased consumption of primary producers (Figure 25), containing high concentrations of  $^{65}$ Zn shown later. The final  $^{65}$ Zn specific activity in the fish was higher than the zooplankton specific activity which was higher than water specific activity. A possible explanation for this is that  $^{65}$ Zn was removed rapidly from the water by components such as the primary producers which have a long biological half-life for Zn, up to 100 days (Gutknecht, 1965). The zooplankton accumulated the  $^{65}$ Zn while maintaining about the same total In concentration at the end of the study period as before

spiking. This produced higher specific activity in the zooplankton than in the water.

## Sediment

Isotope concentrations were measured in the sediment before any isotope addition, at mid-study and at the end of the study period. The isotope concentrations and specific activities of the sediment in all four ponds are given in Table 6. The Cs isotope concentrations, in all situations where no Cs was added, were below detectable limits. No specific activity could be calculated where one or both of the isotope concentrations of Cs or Zn were below detectable limits.

The <sup>134</sup>Cs specific activities of the sediment, throughout the study, in all ponds were lower than the calculated specific activity for the water. This is another indication that some stable Cs was present in the system before the isotopes were added.

Low values of  $^{65}$ Zn specific activity were measured in the sediment, due to the high concentrations of stable Zn present in the sediment before spiking.

Initially core samples were taken of the entire depth of the sediment and split into 2 cm sections for analysis, to determine if any difference in isotope concentrations occurred with depth. Before spiking, there was no difference in Zn concentrations with depth. After spiking the depth distribution of the radioisotopes was measured. The radioisotope portion of the spike was concentrated in the upper 2 cm of the sediment, as shown in Table 7.

Isotope concentrations and specific activities of sediment samples on a dry weight basis. Means of five samples  $\pm$  1 S. E. 9 Table

	Total Cs mg/kg	134 <sub>Cs</sub> pCi/g	134 <sub>Cs S.A.</sub> nCi/mg	Total Zn mg/kg	65 <sub>Zn</sub> pC1/g	65 <sub>Zn S.A.</sub>
POND 1						
Pre-spike Mid-study End-study	B.D.* 1.34±0.14 2.03±0.20	B.D. B.D. 127.96±54.61	B.D. B.D. 58.02±20.46	44.75±5.34 61.46±7.74 83.34±9.65	B.D. B.D. 142.77±54.57	B.D. B.D. 1.53±0.40
POND 2						
Pre-spike Mid-study End-study	B.D. B.D.	в.р. В.р.	B.D. B.D.	52.78±6.54 44.69±2.77 55.46±4.98	8 B B C C C C C C C C C C C C C C C C C	B.D. B.D.
POND 3						
Pre-spike Mid-study End-study	B.D. 0.95±0.21 1.96±0.56	B.D. 149.95±75.50 208.52±72.14	115.07±39.89 136.00±25.48	47.62±9.50 60.65±7.53 54.75±6.78	B.D. 353.35±124.92 323.17±87.96	B.D. 5.38±1.10 5.80±1.65
POND 4						
Pre-spike Mid-study End-study	B.D. B.D. 3.64±0.57	B.D. B.D. 553.06±182.87	B.D. B.D. 145.14±25.25	42.49±2.03 64.28±4.14 70.70±9.75	B.D. B.D. 411.02±66.98	B.D. B.D. 6.65±2.00
	!!!					

\*Below detectable limits for the methods used.

Table 7. Depth distribution of  $^{134}\mathrm{Cs}$  and  $^{65}\mathrm{Zn}$  in sediment cores from Pond 3. Data are means of three samples.

	Mid-	study	End-	study
Depth cm	13 <sup>4</sup> Cs pCi/g	65 <sub>Zn</sub> pCi/g	13 <sup>4</sup> Cs pCi/g	65 <sub>Zn</sub> pCi/g
Top 2	126.51	447.43	179.08	356.38
All samples below	B.D.*	B.D.	В.Д.	B.D.

<sup>\*</sup>Below detectable limits.

## Primary Producers

The isotope concentrations and specific activities of the plants are given in Table 8. The high concentrations of radioisotopes in plant material, suggested when discussing the specific activities of the fish, can be seen in this table. Elodea canadensis was present in large mats, even during the winter months. This longevity would allow a long retention period for the Cs and Zn isotopes. Standing crops of primary producers were measured at the end of the study. These values ranged from 0.25 to 0.52 kg/m², on a dry weight basis, within the range for fertile lakes (Westlake, 1963). Phytoplankton in these ponds was scarce throughout the study period. During the early fall and during the spring some Ceratium sp. was observed in the plankton samples from the ponds.

The <sup>134</sup>Cs specific activities of the primary producers were in general lower than the calculated specific activity for the water from corresponding ponds. This is probably due to the stable Cs that was present in this component prior to spiking.

Primary producers exhibited high  $^{65}$ Zn specific activities relative to the other components of the system. Rapid uptake of the  $^{65}$ Zn added to the water, with little change in stable Zn concentrations during the same period could explain this.

## Isotope Partitioning Between Components

In order to make predictions of specific activities of  $^{134}\text{Cs}$  and  $^{65}\text{Zn}$  in the five components of these fresh water

Isotope concentrations and specific activities of primary producers on a wetweight basis. Means of five samples  $\pm$  1 S.E. Table 8.

	Total Cs mg/kg	134 <sub>Cs</sub> nCi/g	134 <sub>Cs</sub> S.A. nCi/mg	Total Zn mg/kg	65 <sub>Zn</sub> nCi/g	65 <sub>Zn</sub> S.A. nCi/mg
POND 1						
Pre-spike Mid-study End-study	B.D.* 1.45±0.14 5.58±0.35	B.D. B.D. 8.32±2.22	B.D. B.D. 1321.34±202.4	11.83±0.25 15.22±0.90 5.39±0.76	B.D. B.D. 4.49±1.29	B.D. B.D. 962.34±170.64
POND 2						
Pre-spike Mid-study End-study	B.D. B.D.	вв в.о.	в.р. В.р.	5.80±0.66 9.53±0.33 1.98±0.38	вв. В.О. В.О.	B.D. B.D.
POND 3						
Pre-spike Mid-study End-study	B.D. 3.87±0.07 14.67±0.35	B.D. 1.39±0.14 1.78±0.67	B.D. 361.70±33.88 121.56±23.44	4.14±0.57 19.43±3.51 8.04±0.66	B.D. 1.54±0.15 1.21±0.46	B.D. 93.15±11.18 168.79±39.50
POND 4						
Pre-spike Mid-study End-study	B.D. B.D. 60.37±5.20	B.D. B.D. 18.42±0.31	B.D. B.D. 313.15±20.29	18.44±0.25 14.97±0.86 22.04±0.76	B.D. B.D. 10.10±0.39	B.D. B.D. 471.10±8.70

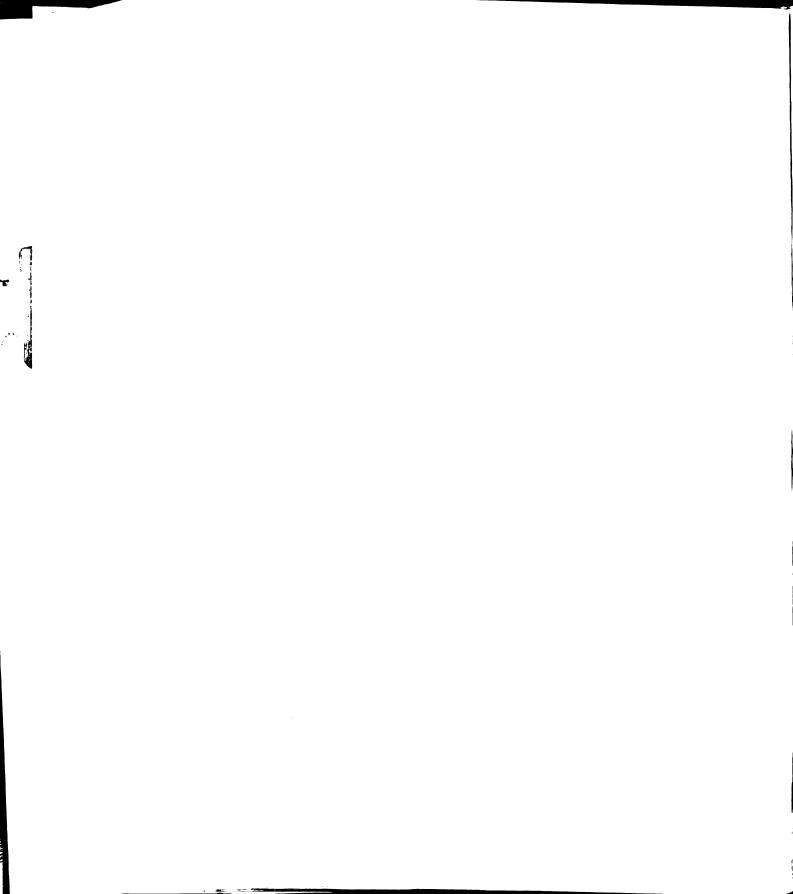
\*Below detectable limits for the method used.

systems, it was necessary to determine the distribution of the stable isotopes in these components. The Cs and Zn isotope concentrations, standing crops of each component and the amount of Cs added were used to calculate the partitioning of Cs and Zn isotopes between the five components. results of the Cs isotope budgeting are given in Table 9. These data are for all four ponds whenever Cs isotopes were detectable. The concrete walls of the ponds behaved similarly to the sediment in accumulating a large fraction of the Cs and Zn isotopes, by sorption. The amounts of Cs and Zn on the walls were therefore included in the sediment portion of the budgets. From 60 to 96 percent of the Cs isotopes were accumulated in the sediment. These values fall within the range reported by Brungs (1967) of 81.38 to 99.39 percent of the Cs added to the ponds being in the substrate after 80 days. The lower value of 60.32 percent was for Pond 4 and this value was caused by the plants in the system still retaining 34.69 percent of the Cs after four months. retention of Cs by macrophytes is moderately long, with values as high as 30 days (Gutknecht, 1965). The retention of radioisotopes by plants could be very important when considering the release of radioactive waste to aquatic communities. Systems with high standing crops of plants might cause a greater hazard, by making the radioisotopes more available to the higher trophic levels such as fish and eventually man. This is demonstrated in these data by the increase in  $^{65}$ Zn in fish near the end of the study in Pond 3

Table 9. Partitioning of Cs isotopes between components, expressed as percents. Numbers in parentheses are radioisotope values.

Time of Sample	Water	Zooplankton	Fish	Sediment	Prim. Prod.
Cs Pond 1					
Pre-spike	B.D.*	B.D.	B.D.	B.D.	B.D.
Mid-study	3.920	0.002	0.213	88.855	6.010
End-study	2.422 (2.428)	0.003 (0.004)	0.454 (0.243)	81.253 (91.638)	15.868 (5.630)
Cs Pond 2	Below de trol pon	tectable limi d)	ts throug	hout the s	tudy (con-
Cs Pond 3					
Pre-spike	B.D.	B.D.	B.D.	B.D.	B.D.
Mid-study	4.124 (5.977)	0.028 (0.003)	0.352 (0.215)		16.064 (8.795)
End-study	1.606 (1.928)	0.002 (0.001)	0.683 (0.238)		6.587 (1.900)
Cs Pond 4					
Pre-spike	B.D.	B.D.	B.D.	B.D.	B.D.
Mid-study	B.D.	B.D.	B.D.	B.D.	B.D.
End-study	4.135 (4.327)	0.024 (0.007)	0.883 (0.380)	60.315 (70.112)	34.693 (25.174)

<sup>\*</sup>Below detectable limits for method used.



which may be attributed to the increased consumption of plants by the fish during that period.

The results of the Zn isotope budgeting calculations are shown in Table 10. These values were calculated using the total Zn measured in the five components of the system. The fraction of the Zn in the sediment ranges from 49.7 to 98.2 percent, with the low value due to 42.8 percent of the <sup>65</sup>Zn remaining in the plants at the end of the study. Retention of Zn by macrophytes is long, with biological half-times of up to 100 days (Gutknecht, 1965).

Comparing In budgets for Pond 2 (control pond) with the ponds receiving Zn isotopes, it appears that the total Zn was distributed similarly in all four ponds. By the end of the study, a total isotope equilibrium was reached in all four ponds. The  $^{65}$ Zn in Pond 3 at the end of the study was distributed similarly to the total Zn. However, Ponds 1 and 4 show large fractions of  $^{65}$ Zn still in the plants. One obvious explanation is that Ponds 1 and 4 were spiked only four months prior to the end of the study, while Pond 3 was spiked nearly 10 months before the final samples were taken. The time of year the spikes were added might also be important in explaining this. Pond 3 was spiked in late fall, when the macrophyte standing crop and gross primary productivity were decreasing rapidly (Figure 6). This suggests that lower uptake rates of the isotopes could account for smaller fractions of added isotopes in the plant material. During decomposition of the plants, a large fraction of the isotopes in the plants could also be released to the other components of

Table 10. Partitioning of Zn isotopes between components, expressed as percents. Numbers in parentheses are radioisotope values.

Time of Study	Water	Zooplankton	Fish	Sediment	Prim. Prod.
Zn Pond 1					
Pre-spike	2.206	0.004	0.132	95.384	2.274
Mid-study	2.136	0.016	0.089	91.710	6.052
End-study	0.568 (0.331)	0.001 (0.049)	0.111 (1.313)	97.140 (61.441)	2.178 (36.866)
Zn Pond 2					
Pre-spike	0.500	0.002	0.104	96.226	3.173
Mid-study	2.100	0.003	0.129	92.706	5.068
End-study	0.636	0.001	0.134	98.225	0.974
Zn Pond 3					
Pre-spike	1.554	0.001	0.133	95.771	2.540
Mid-study	0.254 (17.224)	0.002 (0.001)	0.094 (1.025)	92.008 (72.658)	7.562 (9.091)
End-study	0.330 (0.090)	0.001 (0.004)	0.107 (1.298)	95.715 (89.443)	3.847 (9.164)
Zn Pond 4					
Pre-spike	1.064	0.023	0.123	88.352	10.439
Mid-study	2.096	0.010	0.117	92.270	5.507
End-study	0.938 (6.860)	0.001 (0.019)	0.118 (0.598)	89.203 (49.726)	9.793 (42.797)

the system. The second spiking period, in January, when Ponds 1 and 4 received <sup>65</sup>Zn, was a period when gross primary productivity was low. In the spring, when gross primary productivity was increasing rapidly and plants were increasing in biomass, an increased uptake of isotopes probably took place. Considering these processes, it can be concluded that the time of year the isotopes enter the system would affect the length of time necessary to achieve a specific activity equilibrium.

# Predictions and Statistical Comparisons of Specific Activity

Predicted 134Cs and 65Zn concentrations were calculated assuming that the radioisotopes of Cs and Zn would be distributed similarly to the stable isotopes after a comparable period of equilibration. Stable Cs and Zn budgets, after the first four months, from Pond 1 were used to predict the radioisotope concentrations. The predicted radioisotope concentrations were divided by the total isotope concentrations of Cs and Zn in the components of Pond 1, at the end of the first four month period. These predicted specific activities are given in Table 11. Statistical comparisons of the predicted specific activities and observed specific activities in Table 11, from Pond 1 at the end of the study, were made. The results of these comparisons are summarized in Table 12. Table 12 also includes results of comparisons of predicted specific activities from Pond 1 with observed specific activities from Ponds 3 and 4.

A reiteration of the inherent problems in statistical comparisons of derived variables, such as specific activity,

Table 11.		l observed speci values ± 1 S.	fic activities E.	for components	Predicted and observed specific activities for components of Pond 1 in nCi/mg. Means of five values $\pm$ 1 S. E.
Predicted					
Isotope	Water	Zooplankton	Fish	Sediment	Primary Producers
134 <sub>Cs</sub>	399.65±61.07	436.70±48.52	417.84±43.81	418.75±43.98	414.73±25.43
$65_{\mathrm{Zn}}$	37.91±11.16	41.27±11.52	32.84± 2.93	38.13± 0.40	39.77± 4.69

Observed					
Isotope	Water	Zooplankton	Fish	Sediment	Primary Producers
134 <sub>Cs</sub>	44.79±23.64	1115.21±253.50	234.66±60.74	58.02±20.46	1321.34±202.40
u <sup>2</sup> 59	3.52± 0.69	412.89±138.50	62.71±13.82	1.53± 0.40	962.34±170.64

Comparisons of Summary of statistical comparisons of specific activity data. predicted and observed specific activities. Table 12.

	Water	Zooplankton	Fish	Sediment	Primary Producers
Pond 1 pred	Pond 1 predicted vs. Pond 1 observed	l observed			
134 <sub>Cs</sub>	A P > .75	A P = .15	A P = .1	R P < .002	R P = .02
u <sup>2</sup> 59	A P = .09	R P = .04	A P = .2	R P < .002	R P < .002
Pond 1 predicted	vs. Pond	3 observed			
134 <sub>Cs</sub>	A P = .5	A P > .75	R P = .02	R P < .002	R P < .002
65 <sub>Zn</sub>	R P < .002	A P = .09	R P < .002	R P < .002	R P = .006
Pond l predicted	dicted vs. Pond	4 observed			
134 <sub>Cs</sub>	A P > .75	R P < .002	R P < .002	R P < .002	R P = .02
$65_{\mathrm{Zn}}$	A P = .15	RP < .002	A P = .1	R P < .002	R P < .002

= Rejection of the null hypothesis with a given probability of type 1 error.

K

Evidence indicates that there is no significant difference in specific activities with a given probability of type 1 error. II A

is in order at this point. When calculating a ratio from two sets of independently measured variables the variation of the independent variables is combined in the variance of the ratio. Thus, when the variance of a number of derived variables is calculated, the variance of the ratio is higher than the variation of either of the two independent variables. High variability in measurements leads to insensitivity in statistical comparisons. Heterogenous variance also causes a loss of statistical sensitivity, because it necessitates the use of approximate degrees of freedom for the critical values. Deviations from normality can cause heterogenous variance. This was probably the case in these data, however sample size was too small (5) to allow any test of normality. Because heterogenous variance was not present in all comparisons, one-way analysis of variance techniques were used.

Considering the above discussion, the situation where means differing by a factor of 2.6 are not shown to be significantly different, with P > 0.05, is understandable. All statistical comparisons were made using  $\alpha$  = 0.05 and two-tailed analysis of variance techniques. Probabilities given in the summary tables are presented as accurately as possible from tables presented by Rohlf and Sokal (1969). The predicted  $^{134}\mathrm{Cs}$  specific activity Table 11, compares favorably with the specific activity calculated for water, at the time of spiking in Ponds 3 and 4 (Figures 9 and 11). The statistical comparisons of predicted  $^{134}\mathrm{Cs}$  specific activity with observed specific activity were insensitive, as established

earlier. Thus, it seems reasonable that there was no significant difference between predicted and observed specific activity for water, zooplankton and fish, but with decreasing probabilities (Table 12). The significant difference between predicted and observed \$134\text{Cs}\$ specific activity for sediment and primary producers were probably due to the stable Cs present in the sediment prespike and the high concentrations of \$134\text{Cs}\$ in the macrophytes.

The results of the predicted and observed <sup>65</sup>Zn specific activity comparisons are given in Table 12. The water and fish specific activity were not shown to be significantly different, however, the probabilities are low in both comparisons. The predicted and observed zooplankton <sup>65</sup>Zn specific activity were significantly different, but at a relatively high probability of Type I error. The predicted and observed specific activity for sediment and primary producers were significantly different with a probability less than 0.002.

Comparisons of Pond 1 predicted <sup>65</sup>Zn specific activities with Ponds 3 and 4 observed specific activities show similar results to comparisons of Pond 1 data (Table 12). The isotopes in Pond 3 had equilibrated for 10 months, while Pond 4 was spiked only four months before the end of the study. Pond 1 received stable Cs and Zn at the beginning of the study and <sup>134</sup>Cs and <sup>65</sup>Zn in the middle of the study. Thus, no two ponds received the same isotope combination at the same time and all ponds must be considered as individual units. In all cases the predicted and observed specific

activity for sediment and plants were significantly different for both  $^{134}\mathrm{Cs}$  and  $^{65}\mathrm{Zn}$ . The differences in results of specific activity comparisons for the other components and the results of comparisons using Pond 1 data, are probably a function of the spiking schedule and inherent differences in the systems involved.

Results are given in Table 13 for observed specific activity comparisons between all components at the end of the study. These comparisons were made to establish the validity of the specific activity hypothesis. If the system were in a true equilibrium, for all isotopes, the specific activities should be equivalent in all components. results demonstrate that, in all cases, specific activities for both <sup>134</sup>Cs and <sup>65</sup>Zn differed significantly between components of each pond. These results suggest that a total equilibrium of isotopes was not achieved, after four months in Ponds 1 and 4 or after 10 months for Pond 3. The time necessary for equilibration of the specific activity of all components may be long, a matter of years rather than months. In this situation, where the radioisotopes were added in one dose, eventually the specific activity of the system will equilibrate. However, in natural systems, where the input of radioisotopes is continuous, a specific activity equilibrium may never be attained. The rate of specific activity equilibration is a function of a number of parameters, including, rates of transfer of isotopes between components and the complexity of the community. Total isotope equilibrium does not imply specific activity equilibrium and thus,

Table 13. Summary of statistical comparisons of specific activity data. Comparisons of all components at the end of the study.

	13 <sup>4</sup> Cs	65 <sub>Zn</sub>
Pond 1	Reject, P < .002	Reject, $P = .012$
Pond 3	Reject, $P = .02$	Reject, $P = .02$
Pond 4	Reject, P < .002	Reject, $P = .01$

predictions of radioisotope concentrations based on total isotope distributions may be erroneous (Patten, 1966).

It has been suggested that in most cases, predictions of radioisotope concentrations based on specific activities, yield conservative estimates (Kaye and Nelson, 1968). Fish are one of the major links between freshwater systems and man. Predicted and observed  $^{134}\mathrm{Cs}$  and  $^{65}\mathrm{Zn}$  concentrations for the fish were compared to determine the conservativeness of the predicted values in this study. For both  $^{134}\mathrm{Cs}$  and  $^{65}\mathrm{Zn}$  the observed concentrations were significantly higher (P < .002) than the predicted values in the fish from Pond 1. This may be a function of fish feeding habits, with an increase in consumption of plants high in both  $^{134}\mathrm{Cs}$  and  $^{65}\mathrm{Zn}$  causing high concentrations in the fish near the end of the study. The non-conservative nature of the predicted values is serious where the hazard to man is being assessed.

#### SUMMARY AND CONCLUSIONS

The results of this study indicate that short term predictions of Cs and Zn radioisotope concentrations based on the specific activity hypothesis are not acceptable. The specific activity hypothesis involves basing predicted radioisotope concentrations on stable isotope distributions. The ratio of radioisotope to total isotope (specific activity) must be the same in each component of the system if a specific activity prediction is to work.

Even with the high variability and inherent problems in comparing derived variables, the specific activities of Cs and Zn in all ponds were significantly different between components. Thus, no specific activity equilibrium had occurred even after 10 months.

Predicted  $^{134}$ Cs and  $^{65}$ Zn concentrations in fish were significantly lower than observed values for fish from the same pond. This is hazardous where predicted concentrations are for fish that are eaten by man.

Thorough mixing of stable and radioisotopes between all components of the system appears to be unlikely on a short term basis. This violation renders the simple model of the specific activity hypothesis ineffective in making accurate predictions of radioisotope concentrations.

The aquatic systems used in this study were simple (few trophic levels) and the distributions of radioisotopes did not match the stable isotope distributions even after 10 months. Thus, in a larger system, such as Lake Erie, with a more complex trophic scheme and with a possible continuous addition of radioisotopes, the system may never reach a specific activity equilibrium.

The use of this method for long term predictions, 10 to 20 years or longer, might work if the radioisotope addition was only for a short period.

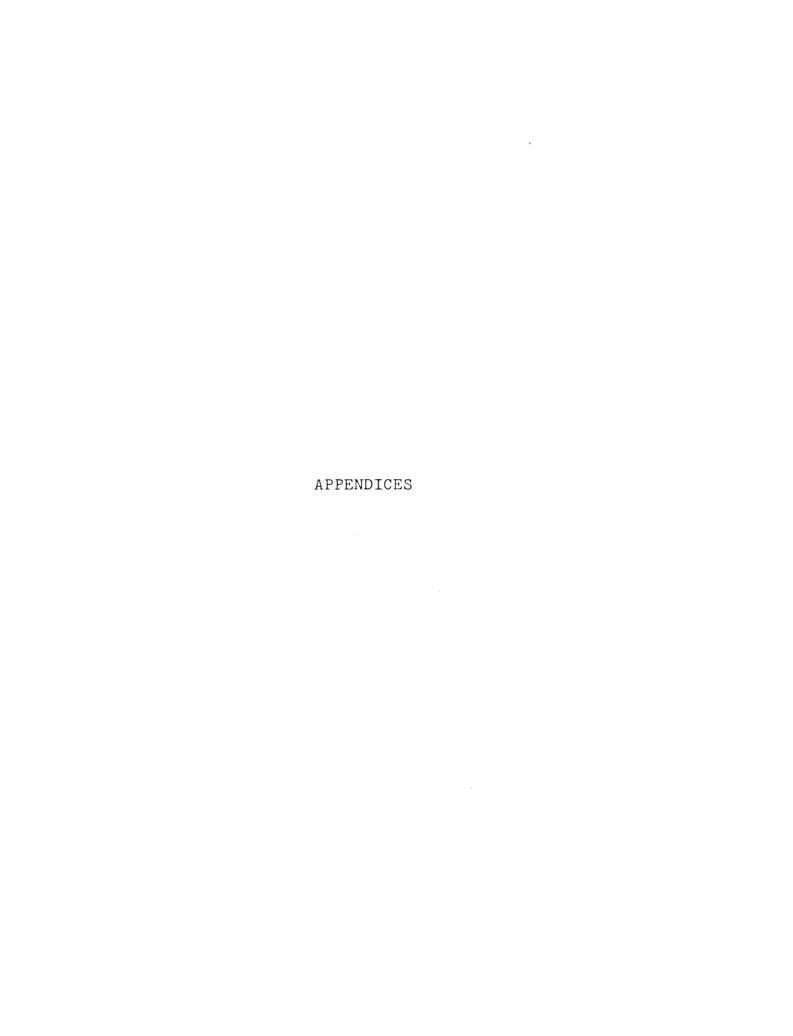
Short term predictions of radioisotope concentrations based on the specific activity concept might be reasonable if a safety factor were used. Multiplying the predicted concentration by a factor of five or ten would allow a conservative margin that is very important where hazards are being assessed.

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#### APPENDIX A

#### METHODS AND MATERIALS

## Pond Enclosures

The temporary greenhouse structure used in this study was designed similar to those used by nurserymen. An excellent description of similar structures is given by Maddax (1973). These enclosures were constructed using 4" x 4" pine timbers fastened to the concrete walls of the ponds with metal plates. Holes 2" in diameter were bored in the timbers at 3 foot centers. The arches were constructed from 1.5" PVC drainage tubing and PVC pressure fittings. These braces placed over the ponds every 3 feet proved to be sufficiently strong to support moderate snow falls usually encountered in this area, however during one extremely heavy snow fall in December 1974 the build up of snow along the edges of the enclosures impeded the snow from falling off the top of the enclosures and caused the arches to bend and finally break. The remainder of the winter after each snow fall the snow was shoveled away from the edges to prevent another collapse.

Polyethylene sheeting 6 mils thickness was initially used but proved to be inadequate during strong winds. Reinforced plastic sheeting with a net of heavy polyethylene between two layers of 4 mil sheeting held up very well. A 200,000 BTU gas furnace was used to maintain temperatures of 1-3° C during the winter months.

## Sample Preparation and Analysis

Total Cs and Zn concentrations were determined using a model 82-800 Jerrell Ash atomic absorbtion spectrophotometer. This instrument was equipped with a red-sensitive photomultiplier in one channel to detect the Cs emission line at 8521 A. An air-hydrogen flame was used for Cs, giving considerably less background noise than air-acetylene and thus lower detection limits. Cs standards were prepared using CsCl in 1.0 N nitric acid and 10.0 mg/kg K was added to these standards and blanks to eliminate K interferences. Zn was analyzed using atomic absorption techniques at 2138.6 A. Standards were prepared using ZnCl2 in 1.0 N nitric acid. Minimum detectable limits for Cs and Zn in these acid solutions were 0.01 mg/kg for both elements. Standards were stored in the same type polyethylene sample bottles as the actual samples. The standards were analyzed periodically to insure no change in concentration had taken place during storage. All atomic absorption techniques used were according to Elwell and Gidley (1967), with only slight modifications as discussed previously.

## Gamma Counting

Activities of  $^{134}\mathrm{Cs}$  and  $^{65}\mathrm{Zn}$  were determined in all samples using standard gamma counting procedures. A Nuclear Chicago 512 channel spectrometer coupled to a 3 inch well

type sodium iodide, thallium activated crystal and sample changer. All samples were counted for  $^{134}\mathrm{Cs}$  and  $^{65}\mathrm{Zn}$  activities, one isotope at a time. The major energy peaks used for these two radioisotopes were sufficiently separated to allow direct counting of each with no problem of spectrum overlap using proper operational setting on the counter. The activities of  $^{134}\mathrm{Cs}$  were determined using the two energy peaks, 0.605 and 0.796 MeV. The  $^{65}\mathrm{Zn}$  activities were measured using the 1.116 MeV energy peak.

Counting times for extremely low activities were determined using the counting time table for 95 percent statistical reliability given by Seelye (1974). All higher activity samples were counted for 10 minutes.

Counter efficiencies were determined for each standard while counting each set of samples. After correcting the observed activities for counter efficiency, the net activity of the sample was corrected for physical decay to the date that the activity of the radioisotopes used for spiking were measured, July 1 for the first period and December 1 for the second period.

#### Water

Water samples, 5 samples 1 liter each, were collected and subjected to the procedure given in Table Al for the determination of the isotope concentrations. Total solids concentrations were also determined in the laboratory on a portion of the samples. Hardness, alkalinity and pH were determined (Standard Methods, 1971) at the pond site.

type sodium iodide, thallium activated crystal and sample changer. All samples were counted for  $^{134}\text{Cs}$  and  $^{65}\text{Zn}$  activities, one isotope at a time. The major energy peaks used for these two radioisotopes were sufficiently separated to allow direct counting of each with no problem of spectrum overlap using proper operational setting on the counter. The activities of  $^{134}\text{Cs}$  were determined using the two energy peaks, 0.605 and 0.796 MeV. The  $^{65}\text{Zn}$  activities were measured using the 1.116 MeV energy peak.

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Water samples, 5 samples 1 liter each, were collected and subjected to the procedure given in Table Al for the determination of the isotope concentrations. Total solids concentrations were also determined in the laboratory on a portion of the samples. Hardness, alkalinity and pH were determined (Standard Methods, 1971) at the pond site.

### Zooplankton

Total seston samples were collected by pumping pond water, at a rate of 19.0 liters per minute (using a Teel, 110 volt centrifugal water pump), through a 7  $\mu$  mesh plankton net and collected in 100 ml polystyrene vials. These samples were placed in the refrigerator overnight. The following morning the upper portion of the sample, containing the living zooplankton, was aspirated off from the samples and the volume of the sample was recorded. Microscope slides were prepared to count and identify the organisms present. The procedure used for preparation of the zooplankton samples for analysis is given in Table A2.

## Fish

All fish samples were processed within 3 days of collection prohibit changes in weight or isotope concentrations during storage. Fish were weighed and total length was recorded. Gut contents were removed and stored in 10 percent formaldehyde solution and the sex of each fish was determined. Stomach contents samples were separated into benthic, planktonic and primary producer components, dired and weighed. The fish were then subjected to the procedure presented in Table A3.

## Sediment

Core samples were collected placed in preweighed counting vials and counted to determine gamma activities of  $^{134}\text{Cs}$  and  $^{65}\text{Zn}$ . The procedure used is given in Table A4.

## Primary Producers

Samples of macrophytes and periphyton were taken for analyses 3 times during the study period. These samples were frozen in plastic bags until time was available for preparation of the samples for analysis according to the procedure given in Table A5. Two gram samples of the primary producers were placed in counting vials and the gamma activity was determined.

Phytoplankton samples were prepared and analyzed similarly to the zooplankton samples as discussed previously.

Table Al. Preparation of water samples for analysis.

- 2. 150 or 250 ml portion, depending on the expected concentration of total Cs and Zn concentrations relative to detection limits, centrifuged at 3500 rpm for 10 minutes.
- 3. 100 or 200 ml of the centrifuged water was placed in a 1200 ml freeze-drying flask and freeze-dired until all water was removed.
- 4. Remaining solid residue was dissolved in 10 ml of 1.0 N nitric acid and saved for total isotope analysis.
- 5. 10 ml of centrifuged water was placed in counting vials and gamma activities were determined.

<sup>1.</sup> Place 10 ml of whole water in a counting vial and count to determine gamma activities.

Table A2. Preparation of zooplankton samples for analysis.

- 1. Filter the sample through 7  $\mu$  bolting cloth.
- 2. Rinse the organisms collected on the bolting cloth into a preweighed counting vial with 50 percent ethanol and determine the gamma activity of the samples.
- Dry the zooplankton samples in open counting vials at 3. 70° C to constant weight, 20-24 hours.
- 4. Determine the weight of the samples.
- Add 1 ml of concentrated nitric acid to each vial and 5. cap tightly.\*
- 6. Place the samples in a boiling water bath for 6-8 hours.
- 7. Remove the caps, add 15 ml of distilled water, recap and heat in the water bath for 3-4 hours and retain this solution for total isotope analysis.

<sup>\*</sup>Digestion method described by Adrian (1971) for fish tissue.

		,

Table A3. Preparation of fish samples for analysis.

- 2. Allow acid to react with fish with no heat applied for 1 hour.
- 3. Reflux at medium heat for 6-8 hours.
- 4. Distill off all but 5-7 ml of the solution in the flask. If only total isotope analyses are to be conducted skip Step 5.
- 5. Remove remaining 5-7 ml from the flask and place in a counting vial, rinsing the flask 3 times with a small amount of distilled water. The total volume in the vial should not exceed 20.0 ml to give proper counting geometry. Gamma activities were then determined on these vials.
- 6. Add 80 ml of distilled water to samples in the boiling flask at Step 4 for total isotope analysis only or to counted samples in 250 ml sample bottle. Heat the sample for 3-4 hours or until all precipitate dissolves and retain this solution for total isotope analysis.

<sup>1.</sup> Place whole fish (3-20 g) in 500 ml boiling flask and add approximately 3 ml of concentrated nitric acid per gram of fish.

Table A4. Preparation of sediment samples for analysis.

- 2. Add 5 ml of concentrated nitric acid to the sample (2-8 g) and 0.1 ml of Dow silicon anti-foaming agent and cap the vial tightly.
- 3. Place the vials in a boiling water bath for 6-8 hours.
- 4. Remove from heat, allow to cool and rinse the sample, with distilled water into a polypropylene centrifuge tube and centrifuge for 10 minutes at 3500 rpm.
- 5. Decant the acid solution, containing the extracted isotopes, from the centrifuge tube into a 100 ml volumetric flask.
- 6. Add 10 ml of distilled water to the sediment in the centrifuge tube and stir for 2 minutes with a plastic spatula.
- 7. Centrigute the sample as before and decant the solution into the volumetric flask.
- 8. Repeat steps 6 and 7 two more times, providing 98-100 percent removal of the isotopes from the sediment.
- 9. Dilute the solution in the volumetric flask to 100 ml with distilled water and retain this solution for total isotope analysis.

<sup>1.</sup> Dry samples at 90 degrees centigrade to constant weight (20-24 hours) and determine the mass of the sample.

Table A5. Preparation of plant samples for analysis.

- 2. Reflux for 6-8 hours at medium heat.
- 3. Distill off all but about 10 ml of the solution in the flask.
- 4. Allow to cool to room temperature and add 90 ml of distilled water.
- 5. Reflux for 3-4 hours or until sample is completely in solution and retain this solution for total isotope analysis by A. A.

<sup>1. 10</sup> g samples wet weight of plant material was placed in a 500 ml boiling flask with 100 ml of concentrated nitric acid and 10 ml of perchloric acid.

# APPENDIX B ADDITIONAL DATA

Table B1. Cs and Zn isotope concentration factors of zooplankton and fish and water concentrations. Pond 1.

Water Cond	centrations			
Date	Total Cs mg/kg	134 <sub>Cs</sub> pCi/g	Total Z mg/kg	
08/14/73 09/04/73 09/25/73 10/16/73 11/06/73 11/27/73 12/17/73 01/02/74 02/15/74 03/07/74 03/28/74 04/18/74	B.D.* 0.086 0.033 0.028 0.023 0.018 0.004 0.003 B.D. B.D. 0.008 0.011 0.002	**    16.23 6.84 1.20 0.65 0.97	0.133 0.130 0.105 0.091 B.D. 0.023 0.077 B.D. 0.019 0.013	    10.19 4.00 1.98 1.09 0.012
Zooplankto Date	on Concentrat Total Cs	ion Factors	Total Zn	65 <sub>Zn</sub>
08/17/73 09/04/73 09/25/73 10/16/73 11/06/73 11/27/73 12/18/73 02/15/74 03/07/74 03/28/74 04/18/74	B.D. 1,093.37 1,387.88 2,636.43 B.D. B.D. 5,257.50 B.D. 2,456.25 B.D. 2,456.25 B.D. 24,065.00	   11,090.57 44,181.29 39,583.33 64,723.08 44,865.98	15,800.00 7,557.14 1,616.92 7,751.43 8,392.42 B.D. 83,834.78 B.D. 27,394.74 839,230.76 2,822.50 48,215.00	28,066.73 37,250.00 44,090.91 39,853.21 3,521,666.60

Table Bl (con't.)

Fish Conce	ntration Fac	tors		
Date	Total Cs	13 <sup>4</sup> Cs	Total Zn	65 <sub>Zn</sub>
08/16/73 09/04/73 09/25/73 10/16/73 11/15/73 11/28/73 01/13/74 02/22/74 03/07/74 04/01/74 04/18/74 05/07/74	B.D. 94.07 318.79 322.14 583.04 566.11 2,360.00 B.D. B.D. 1,925.00 1,956.36 9,145.00	11.44 57.84 1,734.88 3,441.45 4,230.02	1,797.89 251.73 257.38 243.62 467.14 B.D. 414.29 B.D. 1,423.68 3,310.77 3,353.33 7,845.00	11.30 29.44 1,126.41 1,819.17 167,801.66

<sup>\*</sup>Below detectable limits.

<sup>\*\*</sup>Samples not analyzed.

Table B2. Cs and Zn isotope concentration factors of zooplankton and fish and water concentrations. Pond 2.

Date	Total Cs mg/kg	<sup>134</sup> Cs pCi/g	Total Zn mg/kg	65 Zn pCi/g
Water Conce	ntrations			
08/14/73 01/02/74 05/07/74	B.D.* B.D. B.D.	** 	0.004 0.015 0.005	
Plankton Co.	ncentration Fa	ctors		
08/17/73 12/18/73 05/07/74	B.D. B.D. B.D.		40550.00 19190.67 29540.00	
Fish Concen	tration Factor	'S		
08/17/73 02/22/74 05/07/74	B.D. B.D. B.D.	 	8880.00 2548.67 6642.31	

<sup>\*</sup>Below detectable limits
\*\*Samples not analyzed

Table B2. Cs and Zn isotope concentration factors of zooplankton and fish and water concentrations. Pond 2.

Date	Total Cs mg/kg	13 <sup>4</sup> Cs pCi/g	Total Zn mg/kg	65 pCi/g
Water Concent	trations			
08/14/73 01/02/74 05/07/74	B.D.* B.D. B.D.	** 	0.004 0.015 0.005	
Plankton Cond	centration Fa	ctors		
08/17/73 12/18/73 05/07/74	B.D. B.D. B.D.	 	40550.00 19190.67 29540.00	
Fish Concentr	ration Factor	S		
08/17/73 02/22/74 05/07/74	B.D. B.D. B.D.	 	8880.00 2548.67 6642.31	

<sup>\*</sup>Below detectable limits
\*\*Samples not analyzed

Table B3. Cs and Zn isotope concentration factors of zooplankton and fish and water concentrations. Pond 3.

Date	Total Cs mg/kg	13 <sup>4</sup> Cs pCi/g	Total Zn mg/kg	65 <sub>Zn</sub> pCi/g
Water Concer	ntrations			
08/14/73 09/04/73 09/25/73 10/16/73 11/06/73 11/27/73 12/17/73 01/02/74 02/15/74 03/07/74 03/28/74 04/18/74	B.D.* 0.103 0.049 0.031 0.025 0.023 0.004 0.008 0.002 0.006 0.016 0.015 0.002	B.D. 31.1 13.8 6.97 4.52 3.88 2.72 4.04 2.97 2.90 2.62 0.79 0.76	0.012 0.125 0.112 0.085 0.058 B.D. 0.003 0.006 0.015 0.012 0.015 0.027 0.003	B.D. 21.06 9.82 4.70 3.04 2.72 4.72 2.74 2.46 1.52 2.02 1.70 0.005
Zooplankton	Concentrat	ion Factors		
08/16/73 09/04/73 09/25/73 10/16/73 11/06/73 11/27/73 12/17/73 02/15/74 03/07/74 03/28/74 04/18/74	B.D. 7956.41 2932.65 4139.03 4102.40 3770.44 9150.00 B.D. 3155.00 1732.50 B.D. 20215.00	B.D. 9446.90 4273.18 5209.46 6034.07 7212.11 7133.67 3648.14 5991.86 2678.01 18392.40 10947.36	6825.00 4142.40 5017.86 8981.18 20514.14 B.D. 135533.33 84928.00 121233.33 108666.66 7915.92 63560.00	B.D. 513.80 1084.82 6723.70 5590.49 6696.17 2832.22 3662.60 10023.42 8161.68 8441.17 946000.00
Fish Concent	tration Fac	tors		
08/16/73 09/04/73 09/25/73 10/16/73 11/15/73 11/27/73 02/22/74 03/07/74	B.D. 35.63 263.26 364.84 617.60 537.39 5460.00 2791.67 1189.38	B.D. 32.15 242.91 390.76 893.98 820.98 1341.08 1292.55 1326.91	2555.00 316.16 346.07 416.00 611.90 B.D. 2320.00 4262.50 3406.00	B.D. 49.14 326.04 604.77 1387.04 1057.77 1347.56 2163.22 1403.52

Table B3 (con't.)

04/18/74	972.67	3056.96	1766.67	917.70
05/07/74	13135.00	5059.95	12893.33	592656.00

<sup>\*</sup>Below detectable limits

Table B4. Cs and Zn isotope concentration factors of zoo-plankton and fish and water concentrations. Pond 4.

Date	Total Cs mg/kg	134 <sub>Cs</sub> pCi/g	Total Zn mg/kg	65 <sub>Zn</sub> pCi/g
Water Conc	entrations			
08/14/73 12/17/73 01/02/74 02/15/74 03/07/74 03/28/74 04/18/74 05/07/74	B.D.* B.D. 0.089 0.056 0.037 0.021 0.004	**  44.38 2.46 9.13 3.53 1.76	0.009 0.010 0.024 0.076 0.036 0.010 0.009	26.20 14.54 5.34 1.90 0.90
Zooplankton Concentration Factors				
08/17/73 12/18/73 02/15/74 03/07/74 03/28/74 04/18/74 05/07/74	B.D. B.D. 659.55 2855.36 3093.51 47546.19 70552.50	8832.81 109065.00 4994.52 9875.64 18852.27	88584.40 137050.00 174986.84 681700.00 336000.00 100285.55 11830.80	73908.40 76127.92 29213.48 93326.32 34088.89
08/16/73 01/13/74 02/22/74 03/07/74 04/01/74 04/18/74 05/07/74	B.D. B.D. 6.40 22.86 295.95 1059.05 7695.00	 0.84 114.58 152.30 579.15 3167.76	3088.89 1331.67 406.97 702.50 4553.33 5828.89 4577.97	1.22 13.84 134.10 834.37 3141.22

<sup>\*</sup>Below detectable limits
\*\*Samples not analyzed

Mean of Isotope concentrations in fish parts (wet weight) at end of study. five samples  $\pm$  1 S. E. Table B5.

	Total Cs	134 <sub>Cs</sub>	Total Zn	65 <sub>Zn</sub>
	mg/kg	pC1/g	mg/kg	pC1/g
POND 1 Bone Viscera Skin & Scales Muscle	5.85±1.17	2212.4±971.01	22.17±2.740	3702.3±2304.0
	3.31±0.50	1937.1±690.90	7.940±0.650	1500.3±482.93
	4.72±0.68	1943.7±670.70	35.72±2.360	1451.6±441.37
	10.95±1.28	3291.1±1363.4	4.740±0.130	1704.7±685.25
POND 3  Bone Viscera Skin & Scales Muscle	18.85±2.050	4715.5±634.96	50.22±4.170	4023.7±411.00
	15.86±1.110	3575.4±374.48	36.03±11.17	3107.8±226.21
	18.41±2.710	5052.0±690.83	87.19±8.810	5196.0±566.14
	26.26±4.040	5648.5±782.76	9.880±0.710	3532.6±529.66
POND 4 Bone Viscera Skin & Scales Muscle	24.28±3.500	5550.5±879.90	56.65±3.500	2949.0±465.21
	26.66±4.040	6379.5±1179.3	27.78±4.920	4417.8±657.97
	22.81±2.930	5839.8±841.68	76.34±6.820	3062.7±464.07
	28.92±3.680	6523.3±815.36	12.82±3.570	3543.2±680.73

Table B6. Total Zn concentration in relation to fish weight (wet weight).

Sample Number	Whole Fish Wt.	Total Zn Concentration mg/kg
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30	3.31 4.37 4.43 4.49 5.09 5.22 5.89 6.65 7.02 9.55 9.68 9.90 10.67 10.71 11.17 11.70 13.60 13.82 15.50 16.64 17.12 17.15 17.80 20.68 20.79 23.91 32.34 40.12	164.73 171.51 162.34 187.17 175.73 161.27 188.94 177.53 177.86 93.47 35.18 169.30 125.45 109.96 144.40 69.01 122.42 91.72 102.82 91.72 102.82 91.74 955.74 965.69 48.35 68.57 27.90 74.76 42.59 36.12

Table B7. Specific activities in nCi/mg. Means of five samples  $\pm$  1 S. E.

Date	134Cs Specific Activity	65Zn Specific Activity		
Zooplankton Pond 1				
02/15/74 03/07/74 03/28/74 04/18/74 05/07/74	* 3124.29±917.70 1115.21±253.50	350.57±170.25 461.73±210.64 11.72±1.80 1627.21±431.22 412.89±138.68		
Zooplankton Pond 3				
09/04/73 09/25/73 10/16/73 11/06/73 11/27/73 12/17/73 02/15/74 03/07/74 03/28/74 04/18/74	366.17±33.02 412.64±14.30 285.48±21.24 271.94±37.13 323.99±18.99 544.83±123.69  2194.04±727.98 377.23±132.60  311.24±77.78	21.44±5.00 22.38±4.94 44.26±5.11 14.81±2.22 36.36±7.71 3.69±0.66 7.16±1.34 16.47±5.94 10.81±1.89 94.01±25.97 28.24±7.36		
Zooplankton Pond 4				
02/15/74 03/07/74 03/28/74 04/18/74 05/07/74	6938.97±758.19 1622.13±112.48 413.55±32.34 604.73±312.46 134.83±29.70	147.38±2.65 50.61±11.01 46.87±2.28 453.28±192.47 237.40±34.73		

<sup>\*</sup>One isotope concentration below detectable limits

Table B8. Specific activities in nCi/mg. Means of five samples  $\pm$  1 S. E.

Date	134Cs Specific Activity	<sup>65</sup> Zn Specific Activity		
Fish Pond 1				
02/22/74 03/07/74 04/01/74 04/18/74 05/07/74	36.29±20.60 54.10±16.83 57.21±9.73 95.19±15.49 234.66±60.74	4.50±2.39 4.45±1.28 45.10±19.65 48.13±13.84 62.71±13.82		
Fish Pond	3			
09/04/73 09/25/73 10/16/73 11/15/73 11/27/73 02/22/74 03/07/74 04/01/74 04/18/74	271.56±18.47 257.48±3.70 237.80±7.56 267.67±13.74 261.59±15.91 358.82±17.04 243.19±27.94 181.78±15.65 157.72±13.10 144.06±7.39	26.12±2.71 80.67±12.12 79.31±9.36 120.05±28.30 93.78±4.72 94.24±14.6 70.45±8.84 55.02±4.81 32.20±5.16 77.32±8.16		
Fish Pond 4				
02/22/74 03/07/74 04/01/74 04/18/74 05/07/74	63.94±9.40 191.34±29.43 118.99±11.75 82.93±26.32 174.96±13.83	1.02±0.14 8.05±3.30 14.52±3.10 30.03±1.86 57.49±10.25		

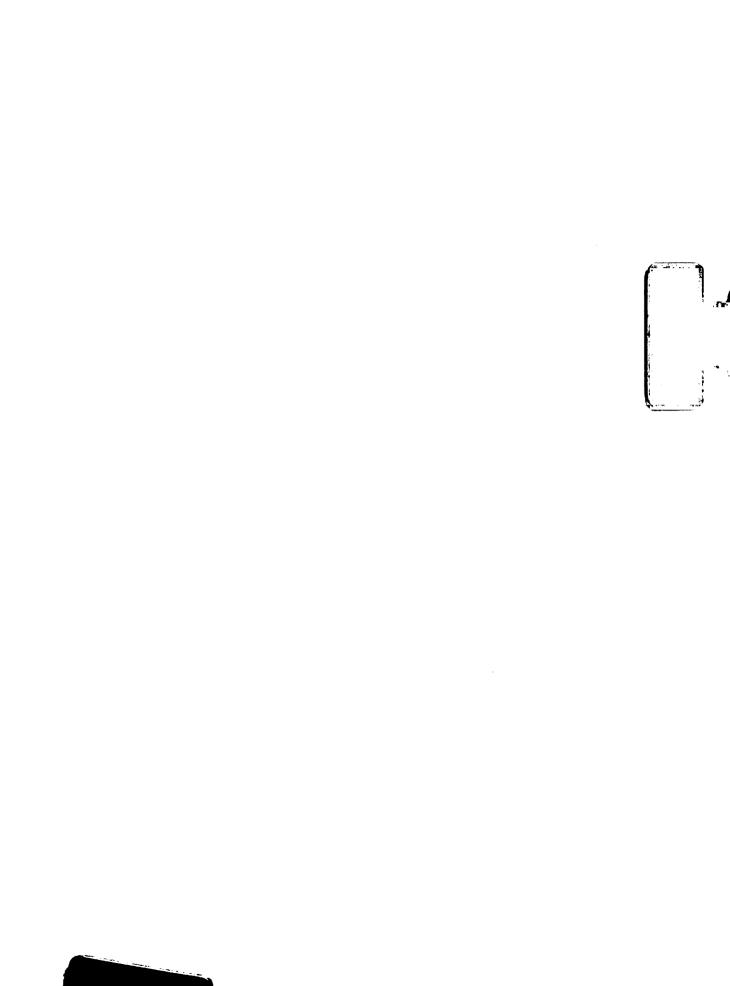
Table B9. Specific activities in nCi/mg. Means of five samples  $\pm$  1 S. E.

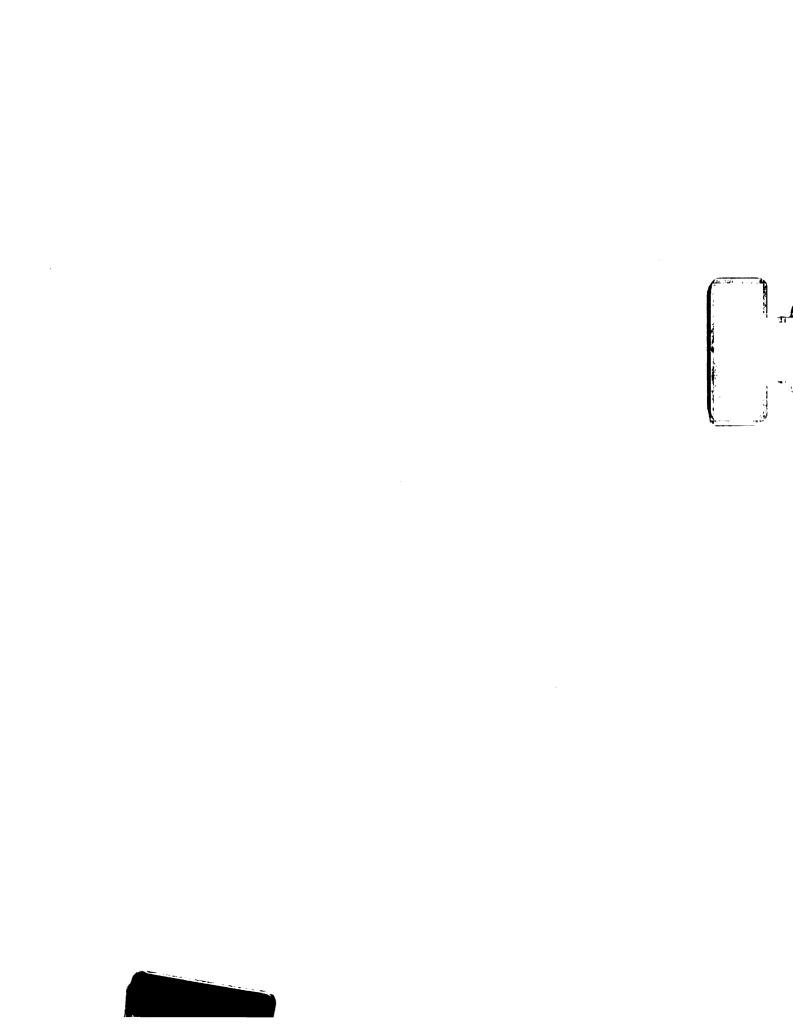
Date	<sup>134</sup> Cs Specific Activity	<sup>65</sup> Zn Specific Activity
Water Pond	1 1	
02/15/74 03/07/74 03/28/74 04/18/74 05/07/74	* 133.10±15.72 60.21±10.43 444.79±23.64	214.67±20.84 148.54±18.31 109.68±49.95 3.52±0.69
Water Pond	1 3	
09/04/73 09/25/73 10/16/73 11/06/73 11/27/73 12/17/73 01/02/74 02/15/74 03/07/74 03/28/74 04/18/74	301.95±5.87 283.12±6.46 225.31±5.88 178.03±4.17 170.15±11.36 658.33±72.65 532.80±73.03 733.53±84.09 460.00±29.63 166.04±23.75 54.44±12.86 402.82±33.35	174.74±20.20 88.89±8.11 58.28±11.22 52.88±11.11  1716.67±239.91 565.33±97.17 173.73±40.13 137.00±25.38 169.70±45.62 67.72±15.86 13.28±7.53
Water Pond	3 4	
02/15/74 03/07/74 03/28/74 04/18/74 05/07/74	508.22±13.20 44.43±2.57 250.69±12.24 163.24±2.94 444.03±43.00	347.91±19.90 408.86±36.90 554.75±88.60 224.78±33.48 89.28±20.98

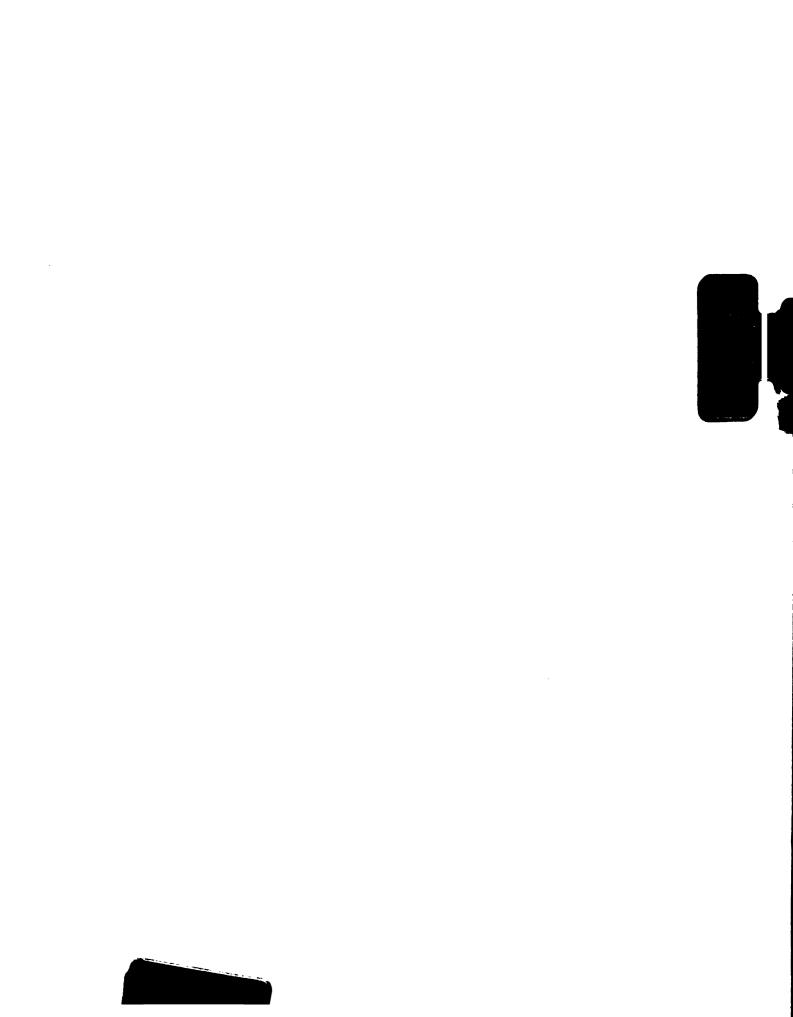
<sup>\*</sup>One isotope concentration below detectable limits

Table Bl0. Specific activities in nCi/mg. Means of five samples  $\pm$  1 S. E.

Pond	Date	13 <sup>4</sup> Cs Specific Activity	65 Zn Specific Activity
Primary	Producers		
1 3 3 4	05/14/74 01/02/74 05/14/74 05/14/74	1321.34±202.40 361.70±33.88 121.56±23.44 313.15±20.29	962.34±170.64 93.15±11.18 168.79±39.50 471.10±8.70
Sediment	;		
1 3 3 4	05/10/74 01/02/74 05/10/74 05/10/74	58.02±20.46 115.07±39.89 136.00±25.48 145.14±25.25	1.53±0.40 5.38±1.10 5.80±1.65 6.56±2.00







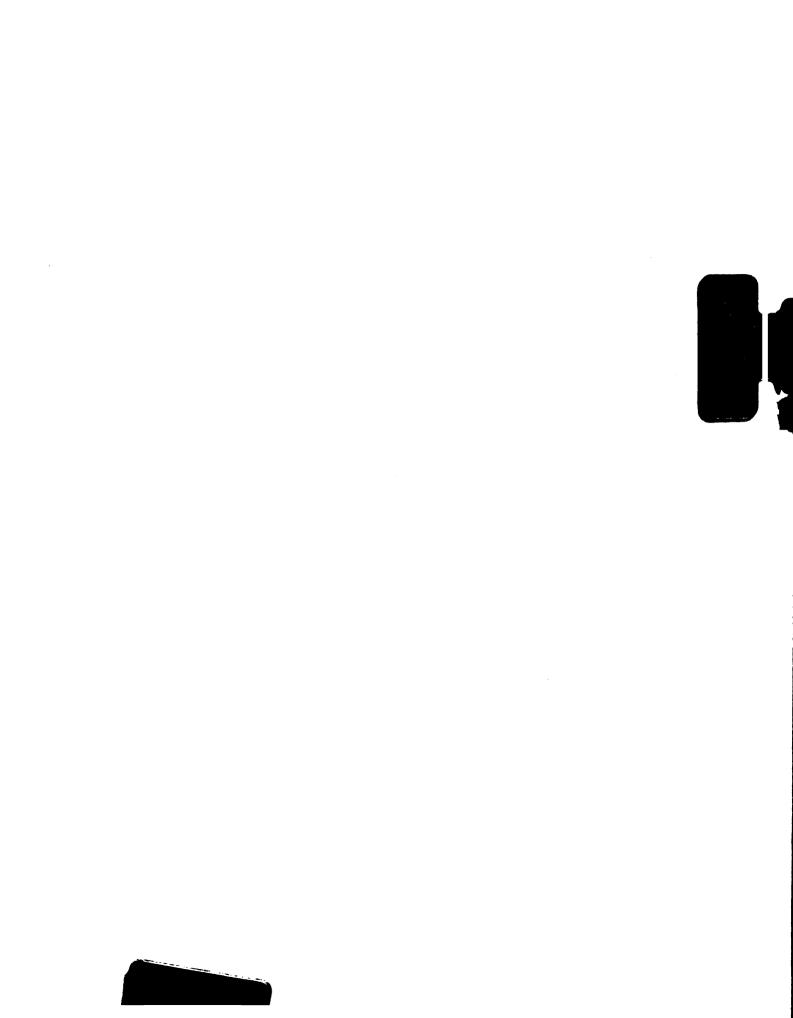


Table B7. Specific activities in nCi/mg. Means of five samples  $\pm$  1 S. E.

Date	13 <sup>4</sup> Cs Specific Activity	65 Zn Specific Activity
Zooplankto	on Pond 1	
02/15/74 03/07/74 03/28/74 04/18/74 05/07/74	* 3124.29±917.70 1115.21±253.50	350.57±170.25 461.73±210.64 11.72±1.80 1627.21±431.22 412.89±138.68
Zooplankto	on Pond 3	
09/04/73 09/25/73 10/16/73 11/06/73 11/27/73 12/17/73 02/15/74 03/07/74 03/28/74 04/18/74	366.17±33.02 412.64±14.30 285.48±21.24 271.94±37.13 323.99±18.99 544.83±123.69  2194.04±727.98 377.23±132.60  311.24±77.78	21.44±5.00 22.38±4.94 44.26±5.11 14.81±2.22 36.36±7.71 3.69±0.66 7.16±1.34 16.47±5.94 10.81±1.89 94.01±25.97 28.24±7.36
Zooplankto	on Pond 4	
02/15/74 03/07/74 03/28/74 04/18/74 05/07/74	6938.97±758.19 1622.13±112.48 413.55±32.34 604.73±312.46 134.83±29.70	147.38±2.65 50.61±11.01 46.87±2.28 453.28±192.47 237.40±34.73

<sup>\*</sup>One isotope concentration below detectable limits

Table B8. Specific activities in nCi/mg. Means of five samples  $\pm$  1 S. E.

Date	134Cs Specific Activity	65 <sub>Zn</sub> Specific Activity		
Fish Pond 1				
02/22/74 03/07/74 04/01/74 04/18/74 05/07/74	36.29±20.60 54.10±16.83 57.21±9.73 95.19±15.49 234.66±60.74	4.50±2.39 4.45±1.28 45.10±19.65 48.13±13.84 62.71±13.82		
Fish Pond	3			
09/04/73 09/25/73 10/16/73 11/15/73 11/27/73 02/22/74 03/07/74 04/01/74 04/18/74	271.56±18.47 257.48±3.70 237.80±7.56 267.67±13.74 261.59±15.91 358.82±17.04 243.19±27.94 181.78±15.65 157.72±13.10 144.06±7.39	26.12±2.71 80.67±12.12 79.31±9.36 120.05±28.30 93.78±4.72 94.24±14.6 70.45±8.84 55.02±4.81 32.20±5.16 77.32±8.16		
Fish Pond 4				
02/22/74 03/07/74 04/01/74 04/18/74 05/07/74	63.94±9.40 191.34±29.43 118.99±11.75 82.93±26.32 174.96±13.83	1.02±0.14 8.05±3.30 14.52±3.10 30.03±1.86 57.49±10.25		

Table B9. Specific activities in nCi/mg. Means of five samples  $\pm$  1 S. E.

Date	134 Cs Specific Activity	65 Zn Specific Activity
Water Pond	1	
02/15/74 03/07/74 03/28/74 04/18/74 05/07/74	* 133.10±15.72 60.21±10.43 444.79±23.64	214.67±20.84 148.54±18.31 109.68±49.95 3.52±0.69
Water Pond	3	
09/04/73 09/25/73 10/16/73 11/06/73 11/27/73 12/17/73 01/02/74 02/15/74 03/07/74 03/28/74 04/18/74	301.95±5.87 283.12±6.46 225.31±5.88 178.03±4.17 170.15±11.36 658.33±72.65 532.80±73.03 733.53±84.09 460.00±29.63 166.04±23.75 54.44±12.86 402.82±33.35	174.74±20.20 88.89±8.11 58.28±11.22 52.88±11.11  1716.67±239.91 565.33±97.17 173.73±40.13 137.00±25.38 169.70±45.62 67.72±15.86 13.28±7.53
Water Pond	4	
02/15/74 03/07/74 03/28/74 04/18/74 05/07/74	508.22±13.20 44.43±2.57 250.69±12.24 163.24±2.94 444.03±43.00	347.91±19.90 408.86±36.90 554.75±88.60 224.78±33.48 89.28±20.98

<sup>\*</sup>One isotope concentration below detectable limits

Table Bl0. Specific activities in nCi/mg. Means of five samples  $\pm$  1 S. E.

Pond	Date	13 <sup>4</sup> Cs Specific Activity	65 Zn Specific Activity
Primary	Producers		
1 3 3 4	05/14/74 01/02/74 05/14/74 05/14/74	1321.34±202.40 361.70±33.88 121.56±23.44 313.15±20.29	962.34±170.64 93.15±11.18 168.79±39.50 471.10±8.70
Sedimen	t		
1 3 3 4	05/10/74 01/02/74 05/10/74 05/10/74	58.02±20.46 115.07±39.89 136.00±25.48 145.14±25.25	1.53±0.40 5.38±1.10 5.80±1.65 6.56±2.00

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