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MASTER

FINAL REPORT

ON

CONTRACT NO. AT(11-1)-1803,

THE ENVIRONMENTAL FACTORS AFFECTING

RADIONUCLIDE CONCENTRATION IN TUBIFICIDS,

BETWEEN

ATOMIC ENERGY COMMISSION

AND

WESTERN MICHIGAN UNIVERSITY

Clarence J. Goodnight

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Under the sponsorship of an Atomic Energy Commission contract (No. AT(11-1)-1803), we have been engaged in studying environmental factors affecting radionuclide concentration in tubificids. Several graduate students have been associated with me in this project. These students include:

1. Douglas Gunnison--received M.A. this summer at Western and will be at Colorado State next year;
2. Andre Purmalis--in Peace Corps; and
3. Edward Block--will receive M.A. next year.

The data collected during this study is now in the process of being prepared for publication. A few papers are finished and are being submitted for publication. One of the initial results of the study was the completion of a report, "The role of tubificid worms in the transfer of radioactive phosphorus in an aquatic ecosystem," published in the Proceedings, National Symposium on Radioecology (2nd), 1967, pp. 270-277. Reprints of this paper were distributed with the first annual report of the contract.

This early study indicated the significance of microorganisms in the accumulation of radionuclides by tubificids. As a result of this information, our initial investigation of the concentration of ^{32}P by tubificids began with a detailed study of the accumulation and retention of this radionuclide by microorganisms.

Since the primary source of ^{32}P accumulation in stream food chains is bacteria, we made a detailed analysis of the factors affecting accumulation and retention of ^{32}P by E. coli. This portion of the study

resulted in a report, "The accumulation and retention of radiophosphorus by Escherichia coli." A copy of this investigation was included in the second year report of the project. It is now in press and will be published in the next issue of The American Midland Naturalist. A summary was also presented at the last annual meeting of the Midwest Benthological Society.

Further studies have investigated the accumulation of ^{32}P by algae and their relationship to accumulation and retention in tubificids. This material has been organized into a report which was presented at the Third National Symposium on Radioecology at Oak Ridge (Conference 710501) in May, 1971. A copy of this report is included.

Other studies are now complete on the uptake and retention of other radionuclides by tubificids. In these cases, the data is being checked and the results are being prepared for publication. These studies include a detailed investigation of the radionuclide ^{137}Cs and several other isotopes. Preliminary results of this portion of the study were included in the second annual report of this study.

The effects of radiation on the blood cells of tubificids and on the respiration rate changes of these worms are at present under investigation.

As soon as these studies have been completed, copies of the papers will be submitted.

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THE RELATIONSHIPS BETWEEN ^{32}P ACCUMULATION IN
ALGAE, BACTERIA, AND TUBIFICIDS

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Abstract. Kinetics of accumulation and retention of ^{32}P were determined for unialgal cultures of Chlorella pyrenoidosa in carrier (52 mg $\text{Na}_2\text{HPO}_4/1.$) and carrier-free Knop's solution (modified) using 10 μC $^{32}\text{P}/1.$ as tracer and a Geiger-Mueller counting system. Accumulation in a carrier-free solution was exponential and approached a 90% efficiency of removal within 30 minutes. In the carrier solution, accumulation was approximately linear and did not reach equilibrium within a 27-hour period. Using ouabain as an inhibitor, it was shown that the observed sorption of radiophosphate in carrier-free solution by this alga was independent of metabolic activity. Most loss of the phosphate taken up in a 27-hour interval occurred within one hour in agitated carrier and carrier-free Knop's solution.

Accumulation of ^{32}P by tubificid worms was also studied using similar counting methods. Uptake by these worms was approximately exponential in media with a bacterial source of radiophosphate, and was reduced by addition of chloramphenicol and removal of ^{32}P from solution by algae. Preliminary results indicated that C. pyrenoidosa competed effectively with the worms for inorganic phosphate, and that accumulation of ^{32}P by ingestion of algae among tubificids may be slight. Ecological implications of these results are discussed.

INTRODUCTION

Radiophosphorus (^{32}P) acts as an effective indicator for studying phosphorus transfer through an aquatic ecosystem in the field (Foster 1959, Ball and Hooper, 1963) and under laboratory conditions (Whittaker 1961, Johannes 1964). Under field conditions the availability of this isotope has produced substantially improved estimates of turnover time (Rigler 1956), concentration ratios (Olsen and Foster 1952) and some ecological relationships of aquatic organisms. Under laboratory conditions, particular species have been studied to determine their importance in the transfer of radiophosphate through an aquatic community (Whitten and Goodnight 1967) and to investigate the effect of environmental parameters such as temperature and isotopic dilution (Whittaker 1961) on the rates of transfer. Such laboratory studies are of particular value in determining the ecological relationships involved in phosphate transfer in fresh-water food chains.

The present investigation is one of a series of studies in progress in this laboratory intended to evaluate the role of tubificid worms in radioisotope accumulation, physiological effects of ionizing irradiation in tubificids, and the relationships between these benthic invertebrates and other aquatic organisms. Previous studies (Whitten and Goodnight 1967) have indicated the role of these worms in the transfer of radiophosphate from sediment, bacteria and water to bluegills (Lepomis macrochirus) and bluntnose minnows (Pimephales notatus). The purpose of the present investigation is to study in more detail the

relationship between phytoplankton, bacteria, and temperature as they influence ^{32}P accumulation by tubificids.

METHODS AND MATERIALS

A Chlorella pyrenoidosa culture (unialgal), obtained from Carolina Biological Supply Company, was maintained on Trypticase Soy Agar (TSA) slants at 23°C . under constant illumination. New stock cultures were inoculated and plated for evidence of bacterial contamination monthly. All algae used in this series of experiments were taken from these cultures.

In the initial investigation of ^{32}P accumulation by algae, C. pyrenoidosa was inoculated into 250 ml Bellco culture vessels which were coated with "Desicote" to prevent ^{32}P adsorption onto glass surfaces. Each of these vessels contained 100 ml sterile, carrier-free Knop's solution, modified (KNO_3 , 300 mg/l.; MgSO_4 , 300 mg/l.; $\text{Ca}(\text{NO}_3)_2$, 900 mg/l.; pH 6.1). Each culture, upon reaching late exponential growth phase, was given one microcurie $\text{Na}_2\text{H}^{32}\text{PO}_4$ (Abbott Radiopharmaceuticals) in a negligible volume. A magnetic stirrer provided mixing and aeration. Temperature was held constant at 23°C .; illumination, provided by a bank of fluorescent lights, was also constant. At preset time intervals after radiophosphate inoculation, a 0.1 ml aliquot of the culture was plated on TSA and incubated at 37°C . for 48 hours as a check for bacterial contamination. The culture was then filtered through a 0.45 micron pore size membrane filter, rinsed, and dried in a desiccation chamber for a minimum of 72 hours. After weighing, the algae on the filter were dispersed in 30 ml water by a magnetic stirrer and five 1.0 ml samples of each resulting suspension were placed on

aluminum planchettes and dried for subsequent Geiger-Muller beta counting. The dilution procedure described above was necessary to avoid excessively high counting rates.

In a study of ^{32}P accumulation by this alga in a carrier solution, 5.2 mg Na_2HPO_4 was added to the Knop's solution at the time of addition of 1.0 μC ^{32}P . Subsequent treatment was as described for the carrier-free experiments.

To determine the efficiency of ^{32}P retention by C. pyrenoidosa, carrier and carrier-free cultures of algae were filtered after 27.0 hours of incubation with 10 μC $^{32}\text{P}/\text{l}$. as described above and the algae replaced in sterile carrier and carrier-free Knop's solution, respectively. Determination of ^{32}P retention followed the same procedure as described for accumulation.

The effectiveness of ouabain as a metabolic inhibitor and ouabain's effect on ^{32}P accumulation in C. pyrenoidosa were investigated by adding varying concentrations of ouabain (Sigma Chemicals) and ^{32}P to 1.8 mg (dry weight) algae suspended in Knop's solution in each reaction vessel of a Gilson respirometer. Temperature was maintained at 24°C . with a water bath; rotation rate was 85 rpm. KOH was used to absorb CO_2 . The respirometer readout provided figures convertible to $\mu\text{l. O}_2$ consumed/mg algae/hr. which were recorded at preset time intervals. Accumulation of ^{32}P was recorded simultaneously as described for the previous experiments. Barometric pressure did not vary throughout the duration of this experiment.

In the tubificid uptake experiments, approximately 5000 tubificid worms (Limnodrilus sp.) were obtained from General Biological. These worms were distributed about equally among 24 Carolina culture dishes

(Carolina Biological Supply Company), each containing 100 ml modified Knop's solution. Twelve of these cultures were placed in an environmental chamber at 10° C. and 12 at 20° C. Each group was subsequently treated as follows: After five days of acclimation at these temperatures with daily changes of autoclaved Knop's solution, approximately 4.6×10^7 (hemocytometer count) algal cells were added to each of four bowls, along with 2.0 mg chloramphenicol. A second series of four bowls in each group received 5.0 ml of an Escherischia coli strain B culture (2% glucose in modified Knop's solution, grown at 37° C. for 36 hours). Two mg chloramphenicol were added to the remaining four bowls in each group, and the volume of each bowl was brought to 100 ml with sterile Knop's solution. One microcurie of ^{32}P was added to each bowl. The bowls were stirred to assure mixing and were covered tightly with sterile aluminum foil. Constant side illumination was provided throughout the experiment.

At a preset time all of the worms were removed from one bowl of each treatment described above. At subsequent intervals worms in the remaining bowls were removed in this way. The worms were rinsed in a carrier solution, blotted, weighed, placed in aluminum planchettes, and digested with hyamine hydroxide for 24 hours. Algae or bacteria adhering to the glass of the culture dishes were carefully scraped off with a spatula, and the total plankton filtered through a 0.35 micron pore size membrane filter followed by a rinse. Planchettes and membrane filters were subsequently counted with an end-window Geiger-Muller tube. In this, and in previous experiments, the number of counts was sufficient to assure accuracy within 5%, and counts were corrected for resolution time, geometry, sample absorption, background, and half-life

using methods described in Chase and Rabinowitz (1962). The means of the data obtained in the investigation of tubificid ^{32}P accumulation were treated as suggested for a $2 \times 3 \times 4$ nonreplicated factorial anova design in Sokal and Rohlf (1969). The factors examined were, respectively, two temperatures (10° and 20° C.), three treatments (E. coli, C. pyrenoidosa with chloramphenicol, and chloramphenicol alone) and four times of sampling (24, 43, 67, and 91 hours). The data was transformed to logarithms prior to the analysis.

The presence of cellulolytic bacteria in the tubificid gut was determined by the following procedure: After sterilization of the exterior integument with 95% ethanol, worms were ground in sterile hand homogenizers until no visible tissue remained. Two-tenths of a ml of this homogenate was then plated on cellulose agar (5.0 g NaCl; 0.2 g MgSO_4 ; 1.0 g $(\text{NH}_4)_2\text{SO}_4$; 1.0 g K_2HPO_4 ; 14 g Difco Bacto-Agar in 1.0 l. distilled H_2O) in replicate Bray dishes and petri plates. Incubation was at 16° C. and 35° C. for two weeks; the presence of a translucent halo in the cellulose agar was considered indicative of cellulase production. This method is similar to methods discussed by Siu (1951).

In order to determine if tubificids can produce an endogenous cellulase, worms grown in a Knop's solution with 20 ug chloramphenicol/ml added were homogenized as described above; and the homogenate, with 0.3 mg chloramphenicol/ml added, was filtered through a .45 micron pore size membrane filter. Aliquots of this sterile filtrate were then added to autoclaved cellulose preparations (1.0 mg cellulose/ml H_2O) at 50° C. at pH 6.8 and pH 8.5. Cellulolytic activity was measured with the Glucostat method as used by Meyers et al. (1960) for cellulolytic marine fungi. Controls were included to measure spontaneous

cellulose hydrolysis, preexisting glucose, and glucose resulting from glycogenolysis.

RESULTS

The accompanying graphs (Figs. 1 and 2) show the accumulation of phosphate- ^{32}P by Chlorella pyrenoidosa in carrier-free and carrier Knop's medium. In carrier free Knop's, removal of this isotope from solution is rapid and exponential. In the carrier solution, isotopic dilution results in a linear accumulation which does not reach a plateau with 27 hours of incubation. In carrier and in carrier-free media, most loss of phosphate occurs in the first hour after a change of medium, after which phosphate accumulation and loss are in approximate equilibrium (Figs. 3 and 4). The linear accumulation of ^{32}P is unusual for radioisotope accumulation results, although approximately linear phosphate accumulation has been noted previously for this alga (Kuhl 1962), and is currently receiving further attention in this laboratory.

C. pyrenoidosa, incubated in concentrations of $5 \times 10^{-5}\text{M}$, $5 \times 10^{-4}\text{M}$, and $5 \times 10^{-3}\text{M}$ ouabain (an inhibitor of cation-dependent adenosinetriphosphatases), showed decreased oxygen consumption with increasing ouabain concentration. Mean endogenous O_2 consumption values (as $\mu\text{l O}_2/\text{mg/hr.}$) were: 16, $5 \times 10^{-5}\text{M}$; 11, $5 \times 10^{-4}\text{M}$; 11, $5 \times 10^{-3}\text{M}$; 18, control without ouabain. The control value is similar to values cited in the literature for O_2 consumption by this alga in this temperature range. Preliminary experiments indicated no significant effect of 10 $\mu\text{C } ^{32}\text{P/l.}$ on metabolic activity of Chlorella. Accumulation of ^{32}P did not vary over the concentrations of ouabain and range of metabolic activity given above.

The graphs of tubificid ^{32}P accumulation (Figs. 5 and 6) and those of particulate (plankton) radiophosphate (Figs. 7 and 8) in the same cultures demonstrate several significant effects (see Anova Tables 1 and 2) of environmental variables. Particulate ^{32}P levels in Chlorella pyrenoidosa, Escherichia coli, and control treatments are significantly different. The mean values for ^{32}P accumulation by tubificids are strongly affected by temperature and the type of plankton competing with the worms for ^{32}P (and simultaneously serving as a source of ^{32}P in the food chain). The rate of accumulation of radiophosphate from C. pyrenoidosa by these worms is similar at both temperatures to accumulation in the antibiotic control series, but substantially less (Time x Treatment interaction, Table 1) than accumulation from bacteria. Microscopic observations of tubificid worms and their faeces, however, demonstrate ingestion of both E. coli (as noted by Coler and Gunner 1967) and Chlorella pyrenoidosa by these worms. Although the final ^{32}P concentrations in tubificids are similar at 10° and 20° C., the rate of accumulation is greater (Temperature x Time interaction, Table 1) at the higher temperature.

These graphs and those discussed below were visually fitted. The highest standard error for retention in a carrier solution was less than 30% of the mean; in other experiments, standard error was less than 20%.

The significant time main effect for tubificid radiophosphate accumulation indicates accumulation rate over the time period of sampling. The algae and bacteria, however, were at approximate equilibrium before the first sample was removed. There is no evident effect of temperature on that equilibrium level ($F < 1$).

Lack of ^{32}P accumulation from ingested Chlorella by tubificids initially suggested an inability of these worms to hydrolyze the cellulosic cell wall of this alga. The Glucostat test for enzymatic degradation of cellulose to β -D-glucose uniformly indicated no glucose appearance in the reaction flasks beyond that occurring in controls (ph 8.5). These worms appear to be incapable of producing a cellulase.

A cellulolytic bacterium was, however, repeatedly isolated from the gut flora of tubificids (Tubifex and Limnodrilus) collected from paper mill effluent sediments at Portage Creek, Kalamazoo, Michigan, December, 1970. This bacterium is a one μ gram-negative rod, aerobic and cryophilic. It produces yellow pigmented colonies in the center of a one cm diameter translucent zone that appears within two weeks of incubation at 16°C . on cellulose agar. This bacterium has also been isolated from decaying wood from the same paper mill effluent.

DISCUSSION AND CONCLUSIONS

The accumulation of radiophosphate by Chlorella pyrenoidosa in these experiments, and subsequent rapid loss in radiophosphate-free Knop's medium, indicate (as noted previously and discussed by Gest and Kamen 1948) that phosphate accumulation by this alga takes place largely by adsorption, with a smaller amount by metabolic absorption. Storage of this ion is in loosely-bound or soluble form. ~~Active~~ accumulation of phosphate by C. pyrenoidosa, though known to occur (Kuhl 1968), is masked by a passive accumulation of these ions. Such a passive accumulation of ^{32}P has been noted in the field with net plankton (Cushing and Watson 1968) and accounts for a significant proportion of the rapid initial removal of ^{32}P from water observed in experiments measuring

phosphate accumulation by unicellular plankton in aquarium microcosms (Whittaker 1961).

The investigation of radiophosphate accumulation by tubificids indicates that the primary effect of a 10° to 20° temperature difference on radiophosphate accumulation via a food chain in these worms is an increased rate of accumulation. There is no clear indication in these data of a change in equilibrium ^{32}P concentrations due to this temperature difference.

The difference between the substantial tubificid radiophosphate accumulation in the Escherischia coli treatment and the lower accumulation (similar to the antibiotic control) in the C. pyrenoidosa treatment in the factorial experiment is evidently due to the lack of cellulase in the gut of these worms. This lack results in an inability to digest the cellulosic cell walls of this alga. Although ^{32}P is loosely bound in these algae, it is not readily available for transport across the worm gut epithelium in the absence of cellulose digestion. In this experiment, bacterial synthesis of cellulase was inhibited by chloramphenicol. That the gut microflora normally supplies cellulase is indicated by the isolation of cellulolytic bacteria capable of synthesizing that enzyme from the gut of these worms. In a related example in the literature, a cellulase obtained from the snail gut enzymatically degraded the (alpha) cellulose of C. pyrenoidosa in vitro (Northcote et al. 1958); such snail cellulases were subsequently shown to be of probable microbial origin (Soedigdo et al. 1970).

Bacterial ^{32}P accumulation resulted in increased radiophosphate accumulation by the tubificids, but the algae removed ^{32}P from solution and did not provide the tubificids with ^{32}P . Thus, the algae evidently

compete with the tubificids for available radiophosphate.

The current study suggests that the bacterial flora of tubificid worms may play a role in the digestion of algae containing a cellulosic cell wall and, consequently, affect the transfer of radiophosphate from these algae to tubificids.

Since cellulase in these worms is not endogenous, but of microbial origin, the possibility exists that the range of temperature, pH, and other limnological parameters tolerated by these bacteria limits the distribution of this enzyme among populations of tubificids. The studies of Brinkhurst (1969) and Brinkhurst and Chua (1969) demonstrate that the gut flora of these worms varies seasonally and between genera. The extent to which such variation characterizes cellulolytic species (such as the cryophilic bacterium isolated in this study) will not be clearly understood until a list of these bacteria and the environmental factors restricting their distribution are established.

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LITERATURE CITED

- Ball, R. C., and F. F. Hooper. 1963. The translocation of phosphorus in a trout stream ecosystem. pp. 217-228 in: Radioecology. V. Schultz and A. W. Klement, Jr. (Eds.). Reinhold, New York.
- Brinkhurst, R. O. 1969. Observations on the food of the aquatic Oligochaeta. Abstract. Verh. Internat. Verein. Limnol. 17: 829-830.
- Brinkhurst, R. O., and K. E. Chua. 1969. Preliminary investigation of the exploitation of some potential nutritional resources by three sympatric tubificid Oligochaetes. J. Fish. Res. Bd. Canada 26: 2659-2668.
- Chase, G. D., and J. L. Rabinowitz. 1962. Radioisotope Methodology. Burgess Publishing Co., Minneapolis.
- Coler, R. A., and H. B. Gunner. 1967. Selective feeding of tubificids on bacteria. Nature 216: 1143-1144.
- Cushing, C. E., and D. G. Watson. 1968. Accumulation of ^{32}P and ^{65}Zn by living and killed plankton. Oikos 19: 143-145.
- Foster, R. F. 1959. Radioactive tracing of the movement of an essential element through an aquatic community with special reference to radiophosphorus. in: Marine Biological Applications of Radioisotope Research Techniques. Publ. Staz. Zool. Napoli, 31 (suppl.): 34-62.
- Gest, H., and M. D. Kamen. 1948. Studies on the phosphorus metabolism of green algae and purple bacteria in relation to photosynthesis. J. Biol. Chem. 176: 299-318.

- Johannes, R. E. 1964. Uptake and release of phosphorus by a benthic marine amphipod. *Limnol. and Oceanogr.* 9: 235-242.
- Kuhl, A. 1962. Zur Physiologie der Speicherung Kondensierter Anorganischer Phosphate in Chlorella. *Vortr. Botan. Hrsg. Deutsch. Botan. Ges.* 1: 157-166
- _____. 1968. Phosphate metabolism of green algae. pp. 37-52 in: *Algae, Man and the Environment*. D. F. Jackson (Ed.). Syracuse Univ. Press, Syracuse.
- Meyers, S. P., B. Prindle, and E. S. Reynolds. 1960. Cellulolytic activity of marine fungi. *Tappi* 43: 534-538.
- Northcote, D. H., J. K. Goulding, and R. W. Horne. 1958. The chemical composition and structure of the cell wall of Chlorella pyrenoidosa. *Biochem. J.* 70: 391-397.
- Olsen, P. A., Jr., and R. F. Foster. 1952. Accumulation of radioactivity in Columbia River fish in the vicinity of the Hanford Works. HW-23093.
- Siu, R. G. H. 1951. *Microbial Decomposition of Cellulose*. Reinhold, New York.
- Soedigdo, R., Lie Sien Nio, S. Adiwikarta, and R. C. Barnett. 1970. Cellulase from the snail, Achatina fulica (FER). *Physiol. Zool.* 43: 139-144.
- Sokal, R. R., and F. J. Rohlf. 1969. *Biometry*. W. H. Freeman, San Francisco.
- Whittaker, R. H. 1961. Experiments with radiophosphorus tracer in aquarium microcosms. *Ecol. Monogr.* 31: 157-188.

Whitten, B. K., and C. J. Goodnight. 1967. The role of tubificid worms in the transfer of radioactive phosphorous in an aquatic ecosystem. pp. 270-277 in: Proceedings, National Symposium on Radioecology (2nd). D. Nelson and F. C. Evans (Eds.). Clearinghouse for Federal Scientific and Technical Information, Springfield, Va.

LEGENDS FOR FIGURES

- Fig. 1. Accumulation of ^{32}P in carrier-free Knop's medium by Chlorella pyrenoidosa. Points are mean values.
- Fig. 2. Accumulation of ^{32}P by Chlorella pyrenoidosa in Knop's medium containing 52 mg P/l. Points are mean values.
- Fig. 3. Retention of ^{32}P in carrier-free Knop's solution by Chlorella pyrenoidosa. Points are mean values.
- Fig. 4. Retention of ^{32}P by Chlorella pyrenoidosa in Knop's medium containing 52 mg P/l. Points are mean values.
- Fig. 5. Accumulation of ^{32}P by tubificids in the presence of Chlorella pyrenoidosa, in the presence of Escherichia coli, and with the antibiotic, chloramphenicol, at 20°C . Points are mean values.
- Fig. 6. Accumulation of ^{32}P by tubificids in the presence of Chlorella pyrenoidosa, in the presence of Escherichia coli, and with the antibiotic, chloramphenicol, at 10°C . Points are mean values.
- Fig. 7. Accumulation of ^{32}P by Chlorella pyrenoidosa and Escherichia coli in tubificid cultures compared with tubificids with the antibiotic, chloramphenicol, at 10°C . Points are mean values.
- Fig. 8. Accumulation of ^{32}P by Chlorella pyrenoidosa and Escherichia coli in tubificid cultures compared with tubificids with the antibiotic, chloramphenicol, at 20°C . Points are mean values.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8

Table 1. Anova table for tubificid ^{32}P levels.

	d.f.	Mean Square	F	Significance Level
Main effects				
Temperature	1	.4484	79.645	<.001
Treatment	2	.2616	46.465	<.001
Time	3	.21915	38.925	<.001
Interaction				
Temperature x time	3	.14651	26.023	<.001
Temperature x treatment	2	.00548	.973	*
Time x treatment	6	.04543	8.069	<.025
Time x treatment x temperature	6	.00563		

*Not significant

Table 2. Anova table for total particulate (plankton) ^{32}P .

	d.f.	Mean Square	F	Significance Level
Main effects				
Temperature	1	.07135	.761	*
Treatment	2	.87351	9.311	< .025
Time	3	.33935	3.617	*
Interaction				
Temperature x time	3	.10586	1.128	*
Temperature x treatment	2	.04533	.483	*
Time x treatment	6	.07470	.796	*
Time x treatment x temperature	6	.09381		

*Not significant