OBSERVATIONS ON THE USE OF THE RADIO-ISOTOPE ^{32}P IN THE STUDY OF FOOD UPTAKE BY SOME MAYFLIES AND OTHER BENTHIC MACRO-INVERTEBRATES IN A LABORATORY STREAM ECOSYSTEM

H.J. Schoonbee and J.H. Swanepoel

Research Group for Freshwater Biology
Department of Zoology
Rand Afrikaans University
P.O. Box 524
Johannesburg, 2000
South Africa

ABSTRACT

A laboratory model stream ecosystem was used to study the transfer of the radio-isotope ^{32}P in a food chain, from the water environment through the benthic algae and submerged vegetation to the primary and secondary consumer levels. This type of investigation, in which the nymphs of the mayflies *Baetis harrisoni*, *Neurocaenis discolor*, *Caenis* sp and *Euthraulus bugandensis* were used, revealed the value of ^{32}P (and possibly other isotopes) in the study of not only the rates and quantities of food taken up by mayfly nymphs but also the diurnal activities in feeding by these organisms.

INTRODUCTION

The value of isotopes in the study of the productivity of marine and freshwater environments, including the rate of uptake and accumulation of nutrients such as phosphates by aquatic plants has been demonstrated by a number of research workers (Rigler 1964 ; Lean and Nalewajko 1976). McRoy and Barsdate (1970) showed, by using radio-active phosphorus, that at least 33% of this isotope, absorbed by the roots of the marine macrophyte *Zostera marina*, was again released through its leaves to the surrounding water. Because of severe eutrophication of some rivers and lakes in South Africa and the excessive growths of certain water weeds resulting from it, Vermaak *et al.* (1976) also used ^{32}P to compare the relative uptake and accumulation of phosphates in nine common

aquatic macrophytes.

Algal blooms and luxuriant growths of water weeds unfortunately distract from the fact that the entire food chain is affected by eutrophication. In the same way the utility of isotopes in the study of transfer of nutrients from the producer to the consumer levels in aquatic ecosystems has been largely overlooked.

In the present preliminary investigation, observations were made on the uptake of $^{32}PO_4$ by some selected plants and animals in a freshwater laboratory stream ecosystem.

THE LABORATORY STREAM

Because of the possible dangers involved in releasing radioactive phosphorus into a natural stream, an open air recirculating laboratory stream was constructed. It consisted of a 24.6 m long riffle section, 0.3 m wide and deep, interrupted by two pools each of 1.8 m diameter and 1 m depth. The galvanized steel plate framework of the stream system was lined with replaceable P.V.C. plastic. The stream, which has a capacity of 5300 L was filled with a mixture of tap water and water from a nearby river known to be rich in nutrients from a sewage works effluent. The addition of river water was considered necessary since a growth of algae was required for the algal feeding of the benthic macro-invertebrates which were introduced into the stream. A pump system was fitted to circulate the water continuously through the system. The pool sections were provided with soft bottom sediments whilst different 3 m sections of the riffles were provided with sandy and stony bottom habitats. Material for this was collected from nearby, unpolluted rivers.

ORGANISMS USED

The sago pond weed *Potamogeton pectinatus* was introduced into a 3 m section of the stream as well as in the two pond sections. In order to obtain easily removable algae, a number of glass slides, each 100 cm^2 in surface area,were placed at selected localities in the stream.

During the collection of stones from a nearby river (needed for the stony bottom habitat of the laboratory stream), all associated macro-invertebrate organisms were carefully removed and returned to the stones after their transfer to the laboratory stream. Organisms collected included Hirudinea, Chironomidae larvae, larvae of the caddisfly *Macronema* as well as nymphs of the Ephemeroptera *Euthraulus bugandensis, Neurocaenis discolor, Baetis harrisoni* and *Caenis* sp. The freshwater clam *Corbicula* sp. was introduced to a 3 m sandy bottom habitat similar to that from which it was

originally removed. The mosquito fish *Aplocheilichthys johnstonii* was also released into the stream.

The pump was switched on and the system allowed to circulate for a week prior to the introduction of the isotope in order to allow the organisms to acclimatize.

Observations made during the one week period of acclimatization showed that most organisms survived and showed little signs of discomfort. There was also a marked development of algal growth in the stream itself and on the glass slides placed in the stream during this period.

STREAM CONDITIONS

Regular physical and chemical analyses were made of the stream water during the study. The results of parameters analyzed according to standard procedures for water and waste water (APHA 1971), showed conditions of the stream to be similar to those of clean, slightly enriched streams (Table 1). The water was well

Table 1. Physical and chemical analysis of water in the laboratory stream prior to the addition of the $^{32}PO_4$ isotope.

Parameters*	Mean Values
Temperature °C	22.0
pH	8.6-9.4
Conductivity µS/cm	453.0
Dissolved oxygen	8.1
B.O.D. (5-day)	2.0
Orthophosphate	0.253
Total phosphate	0.470
Ammonia N	0.050
Nitrate N	0.200

* mg/L unless otherwise mentioned

oxygenated throughout the study with little indication of severe microbial activity or much organic material in suspension in the water. The relatively high pH of the water could partly be attributed to the effects of algal growths which developed. It is also an indication of the alkalinity of the water. The values for phosphates, nitrate and ammonia compared closely with those of the river from which the material was collected for the laboratory stream.

METHODS

4.5 mCi of the carrier free isotope $^{32}PO_4$* (specific activity of 86 Ci/mg and mass of 52.3 ng) mixed with the organic dye fluorescein, was released at the head of the stream and the time noted for the water containing the dye and isotope to complete one full cycle of the stream. This was found to take 31 minutes. A further period of two hours was then allowed, i.e. approximately another four cycles of the laboratory stream, before the sampling period commenced. For the next 12 hours all organisms introduced into the stream were sampled every three hours. Two stones together with the algae and macro-invertebrates associated with it, were selected, at random, during each collection period for analysis.

Except for the algae on the stones and the glass slides, all other material collected was thoroughly rinsed with clean water, dried with absorbent paper and the wet biomass of each specimen determined. Samples were then digested in test tubes containining 10 mL 12N HNO_3 in an 80°C water bath. The contents of each test tube were then transferred to a counting vial and its radioactivity determined by Cerenkov counting in a Philips liquid scintillation counter model PW 4510. The required corrections to allow for quenching and the rate of decay of ^{32}P were made for each sample. Counts obtained were then converted to disintegrations per minute (dpm) per g or µg biomass of each organism analysed at a given time.

Since it was difficult to remove all the algae from the stones in the current and from the glass slides without the inclusion of other material, the biomass of the algae was determined according to the quantities of chlorophyll present per unit of stone surface area (as determined using aluminum foil). Algae from the stones and slides were scraped off with a scalpel and the chlorophyll extracted according to Golterman and Clymo (1969).

In order to eliminate the possible effects of colour quenching on samples during the Cerenkov counting process, one mL of

---

* Obtained from the Radiochemical Centre, Amersham

bleaching agent was added to each sample after centrifuging.

RESULTS

From the results on the ^{32}P activity of the organisms studied (Table 2) it is clear that the aquatic weed *P. pectinatus* was very efficient in accumulating the isotope. This plant showed its highest ^{32}P activity for the entire period only two hours after the release of the isotope into the stream. Fluctuations recorded in values of activity for this plant suggest some release of the isotope during day time with definite indications of a further higher release of ^{32}P after dark. The possible translocation of the isotope to the roots of *P. pectinatus* might also be partly resonsible for this decline in activity.

Only if the values of the activity of the algae are converted to dpm/g can one realize how important the benthic algae are in the removal of nutrients such as phosphate from streams. The activity of the algae on the slides was much higher than that on the stones and increased to reach a peak at 2000 h with some indication of a decline at 2300 h. The initial uptake of the isotope by algae on the stones was not only lower than that on the slides but showed a considerably lower level of increase in activity over the study period. Even so, these values, when converted to activity/g biomass, far exceeded those obtained for *P. pectinatus*. Peak values of 32-36 dpm/µg occurred during 2000-2300 h.

One of the first problems encountered during the sampling of macroinvertebrate organisms on the stones in the riffle, was that the removal of two stones per sampling period was not enough to collect all organisms studied. Since there were not enough stones placed into the stream originally, the sampling had to be limited to two stones per collection period. The Hirudinea showed ^{32}P activity for the first time 14 h after the release of the isotope. *Corbicula* sp., which is a filter feeder, already obtained food containing the isotope prior to 1700 h but like *Macronema* sp. did not take in as much food as the actively feeding mayflies and chironomids. The fact that *Corbicula* is a filter feeder might also explain the relatively low value in ^{32}P activity obtained for this species.

The mosquito fish, which feeds on insect larvae, (mainly Chironomidae) only commenced feeding after dark. The isotope activity of 21428 dpm/g at 2300 h suggests that this fish species, which was also collected at 1700 h and 2000 h (Table 2) only started feeding actively after 2000 hr.

Euthraulus bugandensis and *Neurocaenis discolor* were the only two mayfly species sampled during the first three sampling periods. Both already showed isotope activity at 1100 h. Values obtained

Table 2. Time of day, duration of experiment in hours, weather conditions and ^{32}P activity (dpm) for organisms over a period of 14 hours.

Time of Day	1100 h	1400 h	1700 h	2000 h	2300 h
Duration of experiment (hours)	2	5	8	11	14
Weather conditions	cloudy	cloudy	sunshine	night	night
	^{32}P activity of samples during each period in dpm				
FLORA					
Potamogeton pectinatus/g	86800	64250	78550	53850	47050
	36	128	172	343	301
Algae on slides/µg	76	289	283	359	-
	65	212	245	302	-
Algae on stones in current/µg	18	23	28	32	32
	20	17	20	31	36
FAUNA ON STONES IN CURRENT (dpm/g)					
Hirudinea	-	-	0	0	21428
Euthraulus bugandensis	3555	17419	30278	-	-
Neurocaenis discolor	3555	9837	29752	62004	62276
Caenis sp.	-	-	-	-	28360
Baetis harrisoni	-	-	-	92460	136363
Macronema sp.	-	753	-	-	2269
Chironomidae larvae	8288	25806	33026	143316	185660
Corbicula sp.	0	0	928	-	1539
Aplocheilichthys johnstonii	0	0	0	691	1733

over the first eight hours after the release of the isotope suggest that feeding activity in both species increased towards 1700 h and that, in the case of *N. discolor*, feeding activity which further increased after 1700 h, virtually stopped at 2300 h.

Only one sampling of *Caenis* sp. took place at 2300 h. The activity of 23260 dpm/g suggests that this species might not be such an active feeder during the period 1100 - 2300 h.

Baetis harrisoni is one of the most commonly found mayfly species in South African rivers and thrives under organically enriched conditions. Results on its ^{32}P activity, and thus its rate of food uptake suggests that this species is the most active feeder amongst the four species of mayflies studied. At 2300 h *B. harrisoni* had a ^{32}P activity twice that of *N. discolor*.

The caddisfly larvae, *Macronema*, had already obtained food when sampled at 1400 h. Isotope activity recorded for specimens collected at 2300 h suggests that this species was either not receiving sufficient food or did not feed as actively as the mayflies over the same period.

The Chironomidae, which include a large proportion of algal feeders,clearly showed the most active feeding of all macro-invertebrates especially after 1700 h. Values obtained on the ^{32}P activity for this group of organisms indicate that they are feeding mostly during the night.

OBSERVATIONS ON THE ALGAL/MACRO-INVERTEBRATE BIOMASS RATIOS OF ORGANISMS IN THE CURRENT HABITAT

From the results available on the biomass of algae and macro-invertebrates from stones collected during the sampling period, it was possible to calculate the ratio of the algal/macro-invertebrate biomasses for each individual stone. From Table 3 it is clear that the algal biomass (expressed as µg chlorophyll) varies considerably from one stone to another (Column A). There are also similar variations in the macro-invertebrate biomass values from the various stones (Column B). The ratio of algal/macro-invertebrate biomasses showed, almost without exception similar values for each set of stones (Column C). A comparison of values in Columns B and C further shows that there was a decline in macro-invertebrate biomass on the stones between 1100 h and 1700 h after which it increased again to reach maximum values of 13-20 at 2300 h. This implies that some macro-invertebrates leave the stones to feed somewhere else during that part of the day but that they gradually return again to the stones after 1700 h. Values in Column C further suggest that the macro-invertebrates tend to disperse evenly between stones in relation to available (algal) food present.

Table 3. Ratios of algal and macro-invertebrate biomasses collected from the same stones during the sampling period 1100 h - 2300 h.

(A = algal biomass in µg chlorophyll/cm^2 stone surface area;
B = macro-invertebrate biomass in g/cm^2 stone surface area;
C = ratio of chlorophyll/macro-invertebrate biomass µg/g).

Sampling Time	Stones Sampled	A	B($x10^{-5}$)	C($x10^2$)
1100 h	1	0.264	17.0	1.55
	2	0.0596	6.2	0.96
1400 h	1	0.201	8.3	2.40
	2	0.168	7.1	2.40
1700 h	1	0.270	1.5	18.00
	2	0.582	2.4	24.00
2000 h	1	0.120	3.8	3.20
	2	0.174	3.6	4.80
2300 h	1	1.078	20.0	5.40
	2	0.705	13.0	5.40

DISCUSSION

The present study confirms the value of the use of isotopes such as ^{32}P in investigations on the rate of uptake, accumulation and transfer of nutrients in freshwater ecosystems.

The efficient and rapid absorption of phosphates by the sago pondweed *P. pectinatus* within the first two hours of release into the laboratory stream might partly explain why this macrophyte becomes a problem under eutrophic conditions in lakes and rivers. However, results on the uptake and accumulation of the isotope by algae show that these organisms might be far more important than the water weeds in removing nutrients from the abiotic environment and making them available to the rest of the food chain.

This study also showed that minute quantities of nutrients can be followed through the food chain and that this type of

investigation might throw more light on the ecology of the aquatic macro-invertebrate fauna of lakes and rivers. This particularly applies to the nymphs of mayflies which are known to be sensitive to changes in water quality and as such are considered as indicators of prevailing conditions in water bodies.

Although isotopes such as ^{32}P are usually not allowed to be used in natural streams and lakes because of possible dangers to man and animals, their application in a laboratory stream, where work can be done under controlled conditions and where much smaller quantities of isotope are needed to obtain quantitative data, has considerable advantages.

ACKNOWLEDGMENTS

The authors wish to thank The Rand Afrikaans University and the C.S.I.R. for financial support. Our sincerest thanks to Mr. J.J.M. Van Graan for his assistance during the study.

RESUME

On a eu recours à un modèle d'écosystème de cours d'eau en laboratoire pour étudier le transfert du radio-isotope ^{32}P dans une chaîne d'alimentation, depuis le milieu aquatique jusqu'aux niveaux primaire et secondaire de consommation en passant par les algues benthiques et la végétation submergée. Ces recherches, pour lesquelles les nymphes des éphéméroptères *Baetis harrisoni*, *Neurocaenis discolor* et *Euthraulus bugandensis* furent utilisées, montrèrent la valeur du ^{32}P (et peut-être d'autres isotopes) dans l'étude non seulement des taux et des quantités de nourriture absorbée par les nymphes des éphéméroptères mais aussi des activités diurnes relatives à l'alimentation de ces organismes.

ZUSSAMENFASSUNG

Ein Laboratoriums Modell Strom-Ökosystem wurde verwendet, um die Übertragung von Radio-Isotopen ^{32}P in eine Nahrungskette von der Wasserumwelt durch benthische Algen und überflutete Vegetation bis zu den primären und sekundären Verbraucherstufen zu studieren. Diese Art der Untersuchung, bei welcher Nymphen der Eintagsfliegen *Baetis harrisoni*, *Neurocaenis discolor* und *Euthraulus bugandensis* benutzt wurden, offenbarte den Wert von ^{32}P (und möglicherweise anderer Isotopen). Beim Studium der Nahrungsraten und -quantitäten, die von Eintagsfliegen aufgenommen wurden, und auch bei der Beobachtung der täglichen Aktivitäten im Zusammenhang mit der Nahrungsaufnahme erwies sich ^{32}P als besonders nützlich.

REFERENCES

APHA. 1971. Standard methods for the examination of water and waste water. 13th ed. American Public Health Association. Washington, D.C.

Golterman, H.L. and S. Clymo. 1969. Methods for chemical analysis of freshwater. Blackwell, Oxford.

Lean, D.R.S. and C. Nalewajko. 1976. Phosphate exchange and organic phosphorus excretion by freshwater algae. *J. Fish. Res. Board Can.* 33: 1312-1323.

McRoy, C.P. and R.J. Barsdate. 1970. Phosphate adsorption in eelgrass. *Limnol. Oceanogr.* 15: 6-13.

Rigler, F.H. 1964. The phosphorus fractions and the turnover time of inorganic phosphorus in different types of lakes. *Limnol. Oceanogr.* 9: 511-518.

Vermaak, J.F., J.H. Swanepoel and H.J. Schoonbee. 1976. Absorption and accumulation of ^{32}P *Oedogonium* and some aquatic macrophytes. *Water S.A. (Pretoria)*, 2(1): 7-12.