NOTES

An *in Situ* Method for the Measurement of Zooplankton Grazing Rates

ABSTRACT

A radioisotope method is described with which one can measure the rate of ingestion of small particles by zooplankton under natural conditions. Zooplankton is captured and permitted to feed for a short time on trace amounts of ^{32}P-labeled yeast (*Rhodotorula*) automatically released within the specially constructed sampler or grazing chamber; an appropriate experimental time is 5 min, less than the time required for the isotope-labeled food to pass through the gut of the dominant cladoceran grazers. From the uptake of labeled food by the zooplankton retained on a 75-μ net, the filtering rate of the zooplankton community and of individual species can be calculated. High sensitivity, simplicity of measurement, and naturalness of conditions during the experiment are achieved with this experimental technique.

Numerous laboratory studies have shown that filtering rates of zooplankton may be strongly influenced by environmental factors such as temperature (McMahon 1962; Burns and Rigler 1967; Gavlena 1967), concentration of food (e.g. Conover 1956; Monakov and Sorokin 1961; Rigler 1961; Reeve 1963), dimension of food particles (Malovitskaya and Sorokin 1961; McQueen 1970), and chemical nature of the food (Mullin 1963; Reeve 1963; Ivanova 1967). These and other factors fluctuate seasonally and spatially in natural waters, so considerable error may result from extrapolation of laboratory results to field conditions.

The measurement of filtering rates of various zooplankters on a natural food suspension was first attempted by labeling the entire nannoplankton with ^{14}C by adding ^{14}C-Na_2CO_3 to lake water that had been filtered through a fine bolting silk to remove the larger zooplankton and net phytoplankton, reintroducing particular species of zooplankton, and determining their uptake of radioactivity (Nauwerck 1959; Richman 1964). Problems inherent in this method were the lack of assurance that all components of the nannoplankton were equally labeled with ^{14}C, the excessively long experimental period (0.5–4 hr) during which significant egestion may have occurred, and the absence of other zooplankton and larger phytoplankton which may influence the filtering rate. Burns and Rigler (1967) determined filtering rates of *Daphnia rosea* in natural lake water by adding small quantities of highly radioactive yeast cells to unfiltered lake water; however some disturbance, especially to the delicate forms, may result from removing animals from the lake and later testing them in the laboratory. To avoid some of these difficulties Gliwicz (1968) introduced an *in situ* method for estimating grazing rates of zooplankton, in which a special plankton trap was lowered into the lake, and an anaesthetizing agent then released within it. Changes in the abundance of algae, bacteria, and detritus in the poisoned trap were compared with changes in a control trap lacking the poison and net losses in the unpoisoned trap attributed to the effect of grazing by zooplankton. A major contribution of this method was the successful introduction of controlled field experimentation, but the procedure still left several problems unsolved. Defecation and resuspension of undigested food were ignored in the long feeding period (4 hr) required to achieve measurable changes in cell concentration between the two feeding conditions. Long and variable times (up to 10% of the exposure time) were required for the total immobilization of the different zooplankton species by the poison. Finally, identification and enumeration of small algae, bacteria, and detritus is a task that severely limits the number of samples that can be considered.

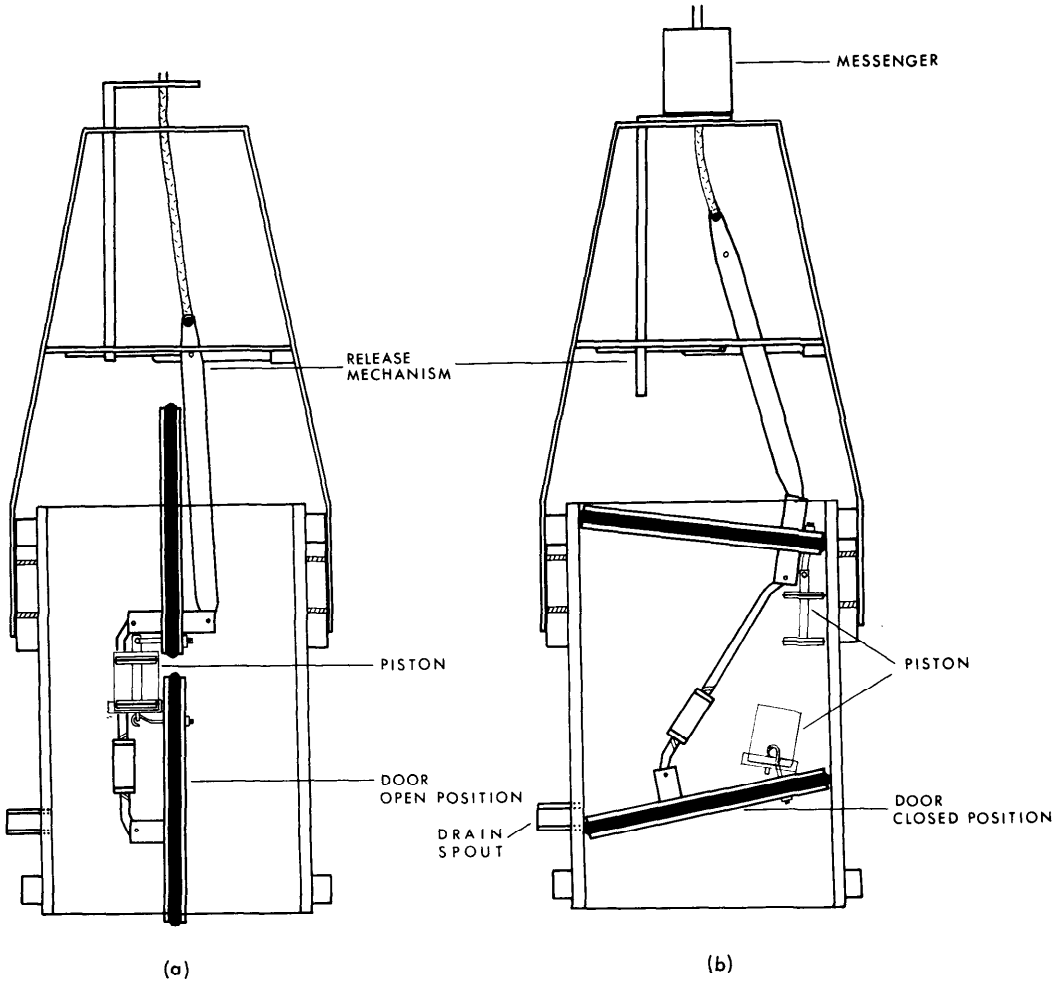

FIG. 1. Diagram (side view) of grazing chamber in (a) open position and (b) closed position.

In this study a radioisotope method for measuring the grazing rate of zooplankton *in situ* was developed. Its applications have been described (Haney 1970). I thank Prof. F. H. Rigler for his valuable advice and Mr. R. Weaver and members of the Department of Zoology Workshop, University of Toronto, for their efforts in constructing the experimental apparatus.

DESCRIPTION OF THE APPARATUS

The application of a radioisotope technique to *in situ* experimentation was made possible by constructing a specially designed water sampler, the grazing chamber (Figs. 1 and 2). The chamber cylinder (23-cm high, 15 cm O.D.) and doors are constructed of clear acrylic (6-mm thickness). The metal superstructure (gravity-release mechanism) is brass. The volume of water contained in the closed chamber is 2 liters. All metal parts within the chamber are stainless steel. The piston (Fig. 1) consists of a stainless steel piston shaft plus a small acrylic cylinder. Watertight seals at the chamber doors and within the piston are achieved with rubber O-rings. The piston-releasing principle was adapted from the plankton sampler of Gliwicz (1968).

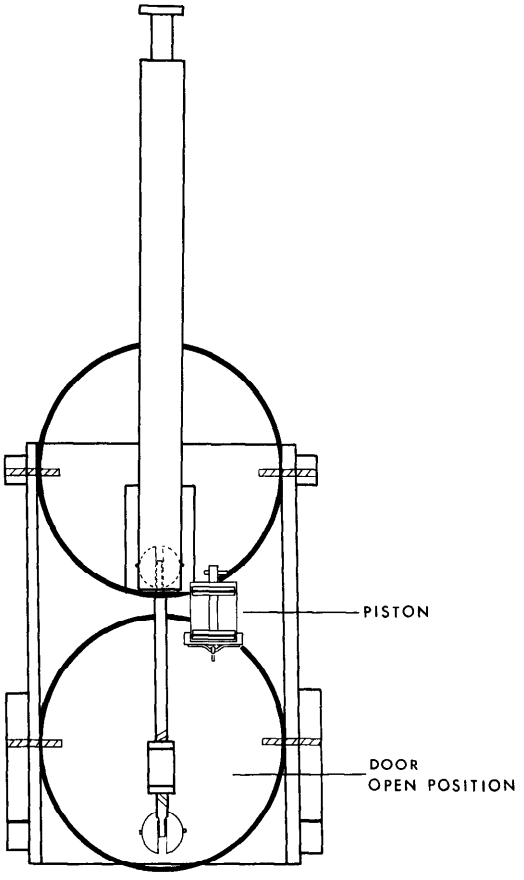

FIG. 2. Diagram (front view) of grazing chamber in open position.

EXPERIMENTAL METHODS

The labeled-food technique used basically follows Burns and Rigler (1967). A small quantity of highly radioactive particles is released into lake water; animals are permitted to feed for a short period in the suspension, removed, quickly killed, and later assayed for radioactivity ingested. Two important limitations of this technique are that the quantity of particles added must be small enough not to alter the natural concentration of particles appreciably and that the feeding time be less than the time required for food to pass through the gut of the animals.

The initial step in the experimental procedure was the preparation of the labeled-food suspension. *Rhodotorula* sp., the yeast used in this study, was isolated from Toronto Harbor, 1967, by Dr. K. E. Chua. Cells of this strain were slightly ovoid and 3.5 μ long. The usefulness of this genus in ^{32}P-labeling experiments has been demonstrated by Burns and Rigler (1967). Axenic cultures of *Rhodotorula* were maintained and grown on a yeast nutrient medium which was modified from that of Burns and Rigler (1967) by reducing the excess phosphate concentration to facilitate the uptake of ^{32}P; no KH_2PO_4 was added and the medium contained 1 g/liter rather than 2.5 g/liter of yeast extract. Labeled yeast was prepared by adding 100–250 μCi of carrier-free ^{32}P-PO_4 (in 0.02 N HCl) to 1 ml of inoculated culture medium. After a 45-hr incubation at room temperature (22 ±2C) under agitation on a wrist-action shaker, the labeled food was centrifuged for 5 min, the liquid decanted, and the cells resuspended in distilled water. Centrifugation and resuspension were repeated four times to ensure complete removal of nonparticulate ^{32}P. Cell concentration was determined by counting 500 or more cells on a hemocytometer. Final activity of the labeled-food suspension was generally 1–10 count min^{-1} per cell. Although fresh labeled foods were normally prepared weekly or biweekly, prepared suspensions of ^{32}P yeast could be held under refrigeration for 2–3 weeks without apparent deterioration of cells or release of radioactivity into solution.

Exactly 1 ml of prepared ^{32}P-labeled yeast was pipetted with an automatic Eppendorf microliter pipette into the small grazing chamber piston (3-ml capacity). Cell concentration was adjusted so that the final concentration of labeled cells in the chamber was 10^3 cells ml^{-1}. The piston was carefully placed inside the opened grazing chamber (Fig. 1a). The chamber was then lowered into the lake to the desired depth and the messenger dropped, activating the gravity-release mechanism. Immediately, as the doors of the chamber closed, the piston was automatically pulled open, and the labeled food was released

inside the closed chamber (Fig. 1b). Tests conducted in the laboratory with colored dyes showed that thorough mixing occurred inside the chamber within 2–3 sec of closure.

The captured zooplankton was allowed to feed for about 5 min; the actual feeding time was measured with a timer. After the feeding period the chamber was quickly retrieved and the zooplankton captured by rapidly filtering the water through a 75-μ Nitex net (high capacity) mounted on a plastic ring (O.D. 50 mm, I.D. 37 mm) fitted in a Millipore 47-mm filter funnel. Time required to empty the chamber was about 15 sec. Timing the feeding period began as the chamber doors closed and terminated as the chamber was completely emptied. After filtration, a small amount of liquid paper paste was applied to the net so that the zooplankton would adhere to it after drying. The net plus ring unit was then placed in a 2-inch (5 cm) aluminum planchet and stored for later radioactivity determination. Sample activity, corrected for background (1.5–3.0 count min^{-1}), was generally several hundred counts per minute per sample providing a standard deviation on total counts per sample of 1% or less (Wang and Willis 1965). No corrections were made for self-absorption by the zooplankton since with high energy beta emitters self-absorption is negligible (Wang and Willis 1965). Wastewater from the experiments was collected in 21-liter plastic carboys. The whole procedure described above was carried out on the lake in a small boat.

Nets containing zooplankton were returned to the laboratory and dried at room temperature for 12–24 hr before counting for radioactivity. Two 50- or 100-μliter samples of the labeled-food suspension were filtered through Millipore membrane filters (47-mm diam, 0.45-μ pore size), which were dried and counted on 2-inch aluminum planchets. Activities from these samples were used to calculate the radioactivity of the experimental food in the chamber.

The grazing chamber technique is sensitive enough to permit the measurement of filtering rates of the individual zooplankton species. For these determinations the zooplankton was captured and permitted to feed on ^{32}P-labeled food in the grazing chamber as described earlier. Following the 5-min experimental feeding period the zooplankton was collected on a 75-μ Nitex net and immediately immersed in CO_2-rich soda water to kill the animals rapidly (Burns and Rigler 1967). All specimens were then washed into 4% formalin. Within 1–3 hr the species were separated and each measured under a binocular microscope. All animals were then transferred with Erwin loops to 32-mm aluminum planchets and arranged within a 10-mm radius of the planchet center to ensure a counting geometry comparable to that of the labeled food on Millipore filters. When sufficient animals were present, from 15 to 25 *Daphnia* or *Diaptomus* and from 25 to 30 *Ceriodaphnia*, *Bosmina*, or *Chydorus* were placed on each planchet. Animals were dried for 24 hr at room temperature and then sprayed three times with a thin coat of Krylon so that the specimens adhered to the planchet. Millipore filters (25-mm diam) containing labeled food for determination of grazing chamber radioactivity were sprayed in an identical manner. The plastic spray caused extremely little attenuation of ^{32}P-beta emissions and thus was useful as a covering for samples of low radioactivity.

Since it has been demonstrated that adult *Cyclops* are primarily predators on other zooplankters and do not filter feed (Fryer 1957; McQueen 1969), adult *Cyclops* were used as a regular check on the possibility that some of the radioactivity measured might have resulted from adherence of ^{32}P label to the body surface rather than from ingestion. Numbers permitting, from 10–20 adult *Cyclops* were picked from each sample and transferred to a planchet. No measurable quantity of radioactivity was recorded for *Cyclops* during this study, indicating that attachment

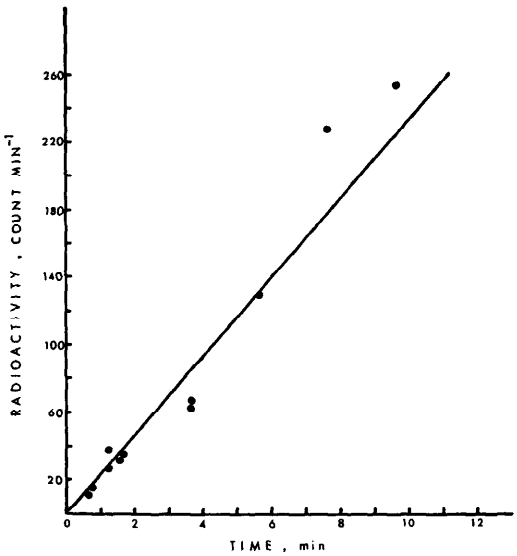

FIG. 3. Time-series 1. Uptake of ^{32}P-labeled yeast by entire zooplankton community. Samples taken from 3.5 m at 14.5C on 7 June 1968.

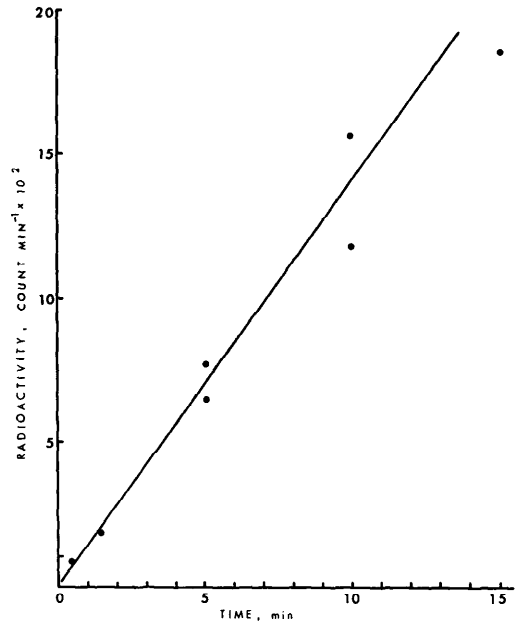

FIG. 4. Time-series 2. Uptake of ^{32}P-labeled yeast by entire zooplankton community. Samples taken from 2.0 m at 17.2C on 19 October 1968.

of ^{32}P or ^{32}P-labeled particles to the body of zooplankton was not an important source of error.

TESTING THE *IN SITU* GRAZING CHAMBER

Before applying the *in situ* grazing chamber method, two of its general aspects were tested. First, it was necessary to confirm that the 5-min feeding period was less than the time required for a radioactive food particle to pass through the gut of a zooplankter. Secondly, if the animals begin feeding immediately and feed constantly thereafter, the rate of food particle ingestion should be relatively constant during the feeding period and this uptake rate should extrapolate to zero activity at zero feeding time.

Zooplankton grazing experiments were carried out through a range of experimental feeding times. Samples for each time-series experiment were taken at a single sampling station and from a single depth to minimize variance between samples due to differences in population densities of the various zooplankters captured. All samples were collected within 2–4 hr beginning at about 1200 hours, according to a randomized scheme to avoid a possible time-related bias. These tests were conducted in Heart Lake, Ontario, a small (17.6 ha) eutrophic lake located about 45 km southwest of Toronto. The labeled food was *Rhodotorula* sp.

The results for grazing by the entire zooplankton community are shown in Figs. 3, 4, and 5. Each point on the graphs represents a single grazing chamber experiment; curves were fitted to the points by sight. All three time-series indicate a constant rate of ingestion of labeled food from about 30 sec (the shortest feeding time), indicating that little or no disturbance in feeding was caused by the closing action of the grazing chamber. All curves extrapolate back to zero activity at zero time. Time series 1 and 2, with maximum feeding times of about 10 and 15 min respectively, fail to show any obvious egestion break in the uptake curve (Figs. 3 and 4). Time-series 3, extending to 45 min of feeding time, clearly illustrates a change in uptake rate after about 10–12 min (Fig. 5). Since the grazing rate of an entire zoo-

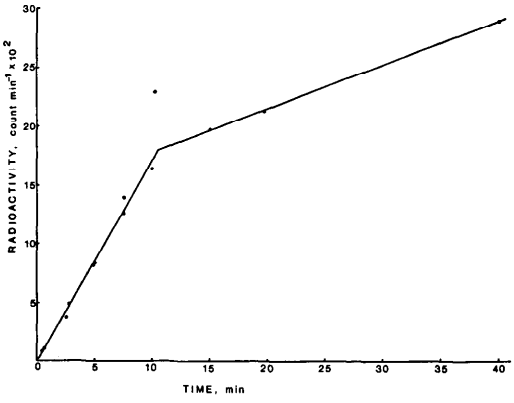

FIG. 5. Time-series 3. Uptake of ^{32}P-labeled yeast by entire zooplankton community. Samples taken from 2.5 m at 21.5C on 11 July 1969.

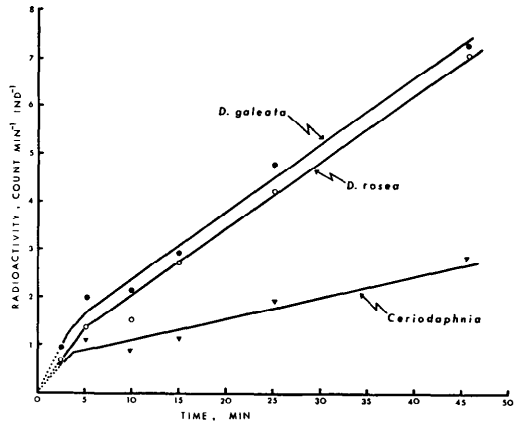

FIG. 6. Time-series 4. Uptake of ^{32}P-labeled yeast by individual species of zooplankton. Samples taken from 2.0 m at 24.0C 14 July 1969. Each point on the graph represents 15–30 animals from a single grazing experiment.

plankton community integrates the feeding of numerous species with a range of different gut-passage times, the appearance of a clear break as opposed to a curvilinear change in the uptake curve suggests the dominance of a single zooplankton species. At the time of time-series 3, the dominant grazers were *Ceriodaphnia quadrangula* and *Daphnia galeata*. Greater diversity of both size and number of species present was exhibited during the time-series 1 and 2; the numerically dominant species were *D. galeata*, *Bosmina longirostris*, *Chydorus sphaericus*, and *Diaptomus oregonensis*. Rotifers, because of their small size and low filtering rates, were not counted.

In a second series of experiments the dominant zooplankters were removed after feeding and the uptake of radioactive yeast was measured for each species separately (Fig. 6). The inflection points on the uptake curves indicate that the gut-passage times for both *D. galeata* and *D. rosea* approximate 5 min. This agrees well with the estimates of Bond (1933) for *D. galeata* (4 min) and Burns and Rigler (1967) for *D. rosea* (6–7 min). Gut-passage time for the smaller *Ceriodaphnia* appears to be slightly less. It may be expected that the time at which egestion begins to show in a time-series will be influenced by the species, their size, the ambient temperature, and the amount of food present. For temperature, time-series 4 (temp, 24C) probably represents the extreme low values of gut-passage time for these species in nature.

In summary, on the basis of these experiments no change in feeding rate resulting from enclosure in the chamber could be detected and a 5-min feeding period was an acceptable experimental time.

EXPRESSION AND CALCULATION OF FILTERING RATES

For filtering suspension-feeding organisms the term "filtering rate" may be defined as the volume of water cleared of suspended particles per unit time (per hour or per day).

From the radioactivity accumulated by an organism, the radioactivity of the feeding suspension and the duration of the feeding period, filtering rate can be calculated as follows:

$$\text{Filtering rate (ml animal}^{-1}\text{ hr}^{-1}) = \frac{\text{count min}^{-1}\text{ animal}^{-1}}{\left[\frac{\text{count min}^{-1}}{(\text{ml grazing chamber suspension})^{-1}}\right]} \times \frac{60}{\text{min feeding time}}.$$

Advantages of this method with short-term feeding experiments have been discussed by Burns and Rigler (1967). Where zooplankton community grazing rates are measured, radioactivity of the total zooplankton collected per sample is substituted for the single animal radioactivity in the equation above.

DISCUSSION

Few investigators have successfully estimated the grazing rate of zooplankton under natural conditions. Gliwicz's (1968) method for measuring zooplankton grazing, although capable of providing important information on the grazing activity of zooplankton, was severely limited in the number of samples that could be taken. The need for information on the changes in biological rate functions throughout the year was indicated in Edmondson's (1961, p. 323) statement that "any techniques which can give information about the rates of reproduction or consumption of any component of a natural community are to be valued, especially when they can be applied repeatedly to follow the seasonal course of development of a community." With the method here described, one can make frequent measurements of the rate of ingestion of small particles by zooplankton under natural conditions. Advantages provided by this technique include: the high sensitivity gained by using radioisotopes, permitting measurement of the grazing rates of sparse populations of zooplankton as well as the filtering rates of very small species of zooplankton; the rapidity and simplicity with which measurements of filtering rates of individual species or the entire zooplankton community can be obtained; the natural conditions maintained during the experiment by using the transparent grazing chamber and the extremely short experimental feeding time of about 5 min, during which time chemical and biological conditions within the chamber remain essentially unchanged; finally, the minimal disturbance to the animals, which feed at the same depth at which they were captured before they are brought to the surface. The method as described uses yeast cells labeled with ^{32}P; however, minor modifications of the technique permit use of a variety of food particles as well as other radioisotopes. Both bacteria and algal cells have also been successfully used as labeled foods, providing a range of particle sizes and types. Although measurements made by this method do not indicate the degree of utilization of small particles by zooplankton, ingestion rates, judiciously used, may provide an initial step toward evaluating the importance of grazing by zooplankton on suspended components in the lake.

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A SIMPLE DEVICE FOR THE SORTING OF LIVING PLANKTONIC COPEPODS

ABSTRACT

A Perspex plankton sorter is described that can be set on the stage of a binocular microscope. A 4-way casing fitted with a 1-way cock allows the sorting of living copepods from a large volume of water without use of a pipette, avoiding thermal stresses.

To study the respiration of Mediterranean planktonic copepods, we had to collect numerous individuals of the same species for a single experiment, most of them being very small. This caused our sorting problem.

We had to identify the copepods under the binocular microscope and then sort them into two different containers. We also had to avoid, as much as possible, any stress liable to modify the metabolism of the copepods, particularly thermal stresses (hence the necessity of working at a constant temperature and by daylight) and mechanical stresses (hence the advantage of avoiding the use of pipettes and the changing of containers).

Bossanyi (1959) used, for preserved-zooplankton counts, a stream of water in which the organisms flow along through a Perspex counting cell beneath the microscope objective. We followed the same principle in the design of our sorting apparatus.

DESCRIPTION OF THE DEVICE

The device is designed as a 4-way casing fitted with a 1-way cock (Fig. 1). It is made of a flat Perspex square that can be set on the stage of a binocular microscope. Two cylindrical drain tubes run at right angles through it from the center of each side. Four rubber tubes can be fitted by means of four glass tube sockets situated at the ends of each of the draintubes. A draintube of the same diameter as those of the casing runs diametrically through the Perspex cock. The diameter of the cock is exactly the diameter of the observing field of the binocular microscope. All the Perspex surfaces inside the observing field are highly polished.

Both tanks, both flasks, and the binocular microscope are set on screwjack stands whose height can be adjusted (Fig. 2).

OPERATING OF THE DEVICE

With the cock turned to position 1 (Fig. 3), the plankton flows along from A to C