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Utilization of dissolved glucose by two copepod species

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Arguments have arisen about whether copepods feed on bacteria or detritus, or possibly on dissolved organic nutrients. In this paper evidence is presented that *Cyclops oithonoides* G. O. Sars, treated with antibiotics, ingests and assimilates dissolved glucose in sterilized brackish water. With glucose solutions of 1 g/l the biological half-life of the carbon ingested is 0.7 days, and with 0.02 g/l it is 1.7 days. Bacterial matter (*Arthrobacter* sp.) is also ingested, but living bacteria pass through the gut more or less undigested. Some organic compounds are assimilated from dead cells in senescent bacterial cultures. *Halectinosoma curticorne* Boeck also retains glucose from solutions of 0.02 g/l, but dies in solutions of 1 g/l; this species seems able to assimilate living as well as dead bacteria (*Arthrobacter* sp.).

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1. Introduction

Information about the food of cyclopid and harpacticid copepods is meagre (MARSHALL 1973), but cyclopid copepods are generally regarded as herbivorous or carnivorous (FRYER 1957). Pronounced differences in food selection have been observed between animals of different sizes; larger species like *Macrocyclops* and *Acanthocyclops* are often predators, whereas smaller species like *Eucyclops* and *Microcyclops* are herbivores (FRYER 1957). In the herbivorous copepods, however, it appears probable that plant cells, although ingested, pass through the gut undigested (MONAKOV & SOROKIN 1959, MONAKOV 1963, MARSHALL 1973).

Cyclops (Mesocyclops) oithonoides G. O. Sars is an important predator of Rotifera during the active pelagic stage of its life cycle (JUMPPANEN 1976). *Cyclops* species have frequently been observed to tear their food open with their mouth parts, and "suck out" the contents. However, the detailed composition of the cell sap ingested is not known. Some investigators hold that copepods are detritus feeders (HEINLE & FLEMER 1975), whereas others suggest that they consume and assimilate bacteria attached to the food ingested (NEWELL 1965, FENCHEL 1972). MARSHALL (1973) considers that bacteria can be used only when aggregated into particles large enough to be retained by the animals.

Some authors have suggested organic substances attached to bubbles rising in the tidal zone may form aggregates suitable for digestion (BAYLOR & SUTCLIFFE, 1963, RILEY 1963). The results presented here suggest that *Cyclops oithonoides* and *Halectinosoma curticorne* Boeck can ingest and assimilate dissolved glucose.

Cyclops oithonoides is a freshwater species which also occurs in the brackish coastal waters of the Gulf of Finland. *Halectinosoma curticorne* mostly remains near the bottom during its whole life cycle, whereas *Cyclops* lives among zooplankton.

2. Material and methods

We started by testing the survival of *Cyclops oithonoides* in a series of pure cultures of bacteria from the Gulf of Finland. Cultures were grown on an agar medium (V medium, consisting of 1.0 g petone, 0.5 g yeast extract, 0.5 g soluble starch, 0.5 g D-glucose, 0.01 g $FeSO_4.7H_2O$, 0.01 g Na_2HPO_4 and 12.0 g agar to which was added 1000 ml of aged brackish water). We then implanted the commonest bacterial species found in the cultures into Petri dishes (diameter 4.5 cm) in filtered and sterilized brackish water. The bacteria were left attached in small clumps, to facilitate their consumption by the copepods. We observed the number of surviving animals and determined TD_{50} (time to 50 % death). In one set of experiments the copepods were fed with fresh bacteria

of all species in the exponential phase of growth, and in another set from a senescent culture with old and dying bacterial cells.

After finding out the bacterial culture in which *Cyclops* and *Halectinosoma* were able to survive, we prepared a V medium with uniformly labelled ^{14}C-glucose for tagging this bacterium (*Arthrobacter* sp., see Table 1). We then transferred the radioactive bacterial cells to sterilized brackish water. In the first set of experiments we transferred bacterial cells in the exponential phase of growth, and in the second set we took senescent cells. As a control we used a solution of pure ^{14}C-glucose in brackish water, treated with antibiotics so that the animals should be free from bacterial cells (cf. Anderson & Stephens 1969). The food concentration (1 g/l) ensured a superabundance of food, as we used only 10—12 animals per Petri dish. The amount of brackish water per Petri dish was about 10 ml. As glucose concentrations of 1 g/l proved poisonous to *Halectinosoma* individuals, we also used concentrations of 0.02 g/l for both *Cyclops* and *Halectinosoma*. Parallel experiments were run in which *Cyclops* was fed with *Scenedesmus acutus* and *S. quadricauda* cells.

The animals were left to feed on bacteria or dissolved organic material for 24 h. This is long enough to guarantee both consumption and assimilation of food particles, as ingested food has a turnover rate of 3—6 h, depending on food quality. We checked this point by feeding the copepods with particles stained with Evans blue in brackish water.

To calculate food consumption and the biological half-life of the radioactive carbon ingested (T_b), we used the formula suggested by Reichle & Crossley (1967):

$$C = k \cdot Q$$

where C is consumption, Q the ^{14}C in the animal's body, and k the elimination coefficient, which is $0.693/T_b$.

Having fed the animals for 24 h on radioactive food we transferred them to a medium containing non-radioactive food in the same concentration. At the time of transfer we took 2—3 individuals to determine the radioactivity of their bodies and gut contents with a Geiger-Müller detector (Wallac RDC-212 meter with GMS-515 detector). We took care to remove any attached radioactive particles by rinsing the animals several times in distilled water. We then sampled *Cyclops* individuals from the non-radioactive medium at 24-h intervals, and were thus able to determine the biological half-life of the material assimilated (T_b in Figs. 1—3). The figures also gave us a rough value for the assimilation efficiency, at the point of intersection of the T_b line with the y axis. The difference between the intersection point and the radioactivity on day 0 is assumed to give the fraction assimilated (see Table 3).

In determining the radioactivity of the food and the absorption of radioactivity into the animal's body we followed the instructions given by Sorokin (1968). The dried food was weighed on a micro-balance, and the weight of the animals was estimated from their volume (Gyllenberg 1973).

We also observed the formation of lipid droplets during the experiment, since it seems that lipid metabolism is connected with assimilation of organic material from the gut (Benson & Lee 1975). This was done by staining the droplets with lipid dyes (see Gyllenberg & Lundqvist 1976), and confirmed by extraction of the lipid material in the animal body with chloroform: methanol 2:1. The amount of lipid was measured as described by Dowgiallo (1968), and the radioactivity in the lipids was determined.

Table 1. Tests on survival of *Cyclops* and *Halectinosoma* offered different kinds of food in sterilized sea water. The table gives mean values for TD_{50} (time in days to death of 50 per cent), standard deviation (S.D.), the number of replicates (N) and reproduction, if any (generations).

Food source	TD_{50}	S.D.	N	Repr.
Cyclops oithonoides				
control (pure sterilized water)	3.5	1.0	6	none
Arthrobacter sp., living cells	2.7	1.2	4	»
Arthrobacter sp., dead cells	14.5	3.2	4	»
Flavobacterium sp., living cells	0.1	0.02	4	»
Flavobacterium sp., dead cells	0.4	0.06	4	»
brown bact.strain, living cells	3.5	0.9	4	»
brown bact.strain, dead cells	4.5	1.3	4	»
glucose medium 1 g/l	39.	8.0	5	»
Scenedesmus acutus Meyen and *S. quadricauda* (Turp.) Bréb.	48.	9.0	3	11
Halectinosoma curticorne				
control	2.2	0.8	5	none
glucose medium 1 g/l	0.7	0.3	4	»
glucose medium 0.02 g/l	14.	2.8	4	»
Arthrobacter sp., living cells	8.5	2.2	4	»

Bacterial cultures were identified by Assoc. Prof. Eva Eklund.

3. Results

From the simple tests of survival it is evident that *Cyclops oithonoides* cannot consume fresh bacterial cells (Table 1), although the tests with radioisotopes confirmed that they are ingested (Fig. 1 B). To some extent both *Cyclops* and *Halectinosoma* can utilize senescent cells (Fig. 1 A, Table 1), possibly in the form of detritus. The parallel experiments in which *Cyclops* was fed with *Scenedesmus* cells showed that algae provide an ample food source, since *Cyclops* was able to survive and reproduce for 11 generations.

The question is now in what form *Cyclops* and *Halectinosoma* ingest their food. The results obtained with the pure ^{14}C-glucose medium seem to throw some light on this question. It is evident that both *Cyclops oithonoides* and *Halectinosoma curticorne* are able to ingest large quantities of dissolved glucose from brackish water. The extremely high body burden in *Cyclops* implies furthermore that the animals are able to ingest glucose and accumlate it in concentrations far

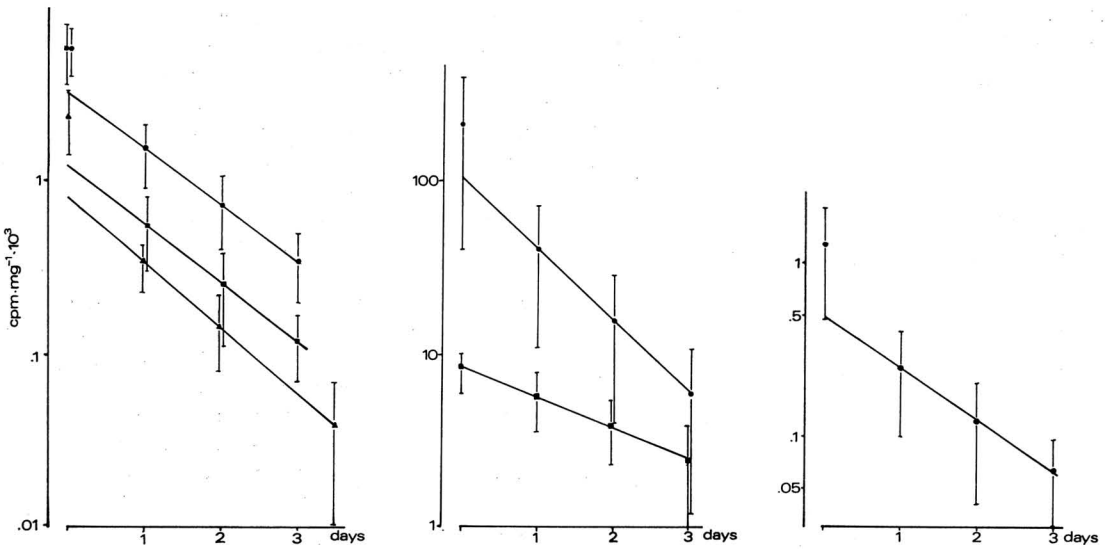

Fig. 1 (left). Radioactivity (cpm) per mg dry weight of *Cyclops oithonoides* individuals fed on cultures of dead bacteria of *Arthrobacter* sp. at 14° C (circles), in cultures of living *Arthrobacter* sp. at 14° C (squares), and with dead *Arthrobacter* sp. (triangles). The time is given as number of days after transfer to non-radioactive medium. The mean and standard deviation for 4 replicates are given for each point on the T_b curve. T_b value for the experiments at 14° C is 0.95 days, for the experiments at 22° C 0.67 days.

Fig. 2 (middle). Radioactivity (cpm) per mg dry weight of *Cyclops oithonoides* and *Halectinosoma curticorne* individuals grown in sterile cultures with glucose. The time is given as number of days after transfer to non-radioactive medium. *Cyclops* individuals in a food concentration of 1 g/l (circles), *Halectinosoma* and *Cyclops* individuals in a food concentration of 0.02 g/l (squares). The mean and standard deviation for 4 replicates are given for each point on the T_b curve. The T_b value for 1 g/l solution is 0.73 and for 0.02 g/l solution 1.7 days.

Fig. 3 (right). Radioactivity (cpm) per mg dry weight of *Halectinosoma curticorne* individuals reared in cultures of living *Arthrobacter* sp., at 14° C. The time is given as number of days after transfer to non-radioactive medium. The mean and standard deviation for 4 replicates are given for each point on the T_b curve. The T_b value for the curve is 1 day.

greater than are present in the surrounding medium (radioactivity of the animals 200 000 cpm/mg as compared with 2100 cpm/mg in the surrounding medium). It seems that in 1 g/l solutions *Cyclops* "sucks up" glucose from the medium.

Furthermore it is clear that a large fraction of what is assimilated as pure glucose is converted into lipids in the animal's body. Table 2 shows a tremendous increase in lipids as compared with control animals from sea water (0.46 ± 0.38 per cent lipids), and chloroform extraction confirmed that 40—60 % of the radioactivity is retained in the lipid droplets. A transformation to lipids would explain how the animals could assimilate and retain radioactive components from the glucose at concentrations far exceeding that in the surrounding medium.

The biological half-life of the ingested carbon is short (0.7—1.7 days, see Figs. 1—3), which emphasizes that the organic substance assimilated is rapidly metabolized.

Table 2. Lipids as percentage of total body volume found in *Cyclops* individuals preserved in 5 % formalin and stained with lipid dyes. The non-radioactive medium was pure sterilized sea water. The values are means for cultures in all media, and standard error (S.E.) for the glucose medium (number of replicates = 4).

days after transfer from ^{14}C medium to non-radioactive medium without food	glucose 1 g/l	living Arthrobacter	dead Arthrobacter
0	14.5 ± 1.7	0.2	0.1
1	4.2 ± 0.5	0.0	0.0
2	3.9 ± 0.5	0.0	0.0
3	0.4 ± 0.2	0.0	0.0

The finding that *Cyclops oithonoides* consumes glucose is supported by visual observations on the animals, which became transparent in glucose solutions of 1 g/l, their guts appearing

Table 3. Parameters of energy flow (consumption and assimilation) as compared with animal live weight. Consumption is given as mg of food consumed per mg of animal live weight per day, mean values and standard errors (S.E.). For details, see text.

Food source	rate of consumption	assimilation efficiency %	mg live wt of animals
Cyclos oithonoides			
living *Arthrobacter* sp.	0.814±0.07	20	0.06
dead *Arthrobacter* sp. 14°C	1.286±0.14	55	0.08
dead *Arthrobacter* sp. 22°C	1.615±0.15	30	0.05
glucose 1 g/l	0.825±0.09	49	0.06
Halectinosoma curticorne			
living *Arthrobacter* sp.	2.00 ±0.24	39	0.04
glucose 0.02 g/l	1.24 ±0.20	100	0.03

white. *Halectinosoma curticorne*, on the other hand, emptied their guts completely in this concentrated glucose medium. There was, furthermore, a significant difference in TD_{50} for *Halectinosoma* as compared with *Cyclops* individuals reared in the same culture (Table 1). The high glucose concentration may be directly responsible for the high mortality of *Halectinosoma* individuals. With concentrations of 0.02 g/l, (1) TD_{50} for *Halectinosoma* increased to about 14 days, (2) all the glucose ingested was assimilated into the animal, (3) the biological half-life of ^{14}C-glucose increased from 0.7 days to 1.7 days for *Cyclops*.

It should be observed that all cultures showed about the same rate of consumption (Table 3).

Cyclops raised on bacterial cultures other than *Arthrobacter* sp., and *Halectinosoma* in a glucose medium of 1 g/l had very low TD_{50} values (Table 1). In the latter case the high concentration of food material was probably directly responsible for the high mortality, having a toxic action on the animals.

4. Discussion

Both *Cyclops oithonoides* and *Halectinosoma curticorne* are freshwater organisms that may form part of the zooplankton in the coastal zone of the Gulf of Finland. *Cyclops* feeds mainly on Rotifers (Jumppanen 1976). In an aquaculture in the laboratory the animals spent most of their time in the bottom layer of the culture medium, and the algal substrate (*Scenedesmus* species) probably offered an ample food source for *Cyclops*. Thus both *Cyclops* and *Halectinosoma* are to a certain extent able to utilize the same substrates for feeding. According to earlier findings on harpacticid copepods, *Halectinosoma* can survice on a bacterial diet for some time (Noodt 1957, Gray 1968, McIntyre 1969).

In contrast to earlier beliefs (Krogh & Berg 1931, Anderson & Stephens 1969, Heinle & Flemer 1975), it is evident that both *Cyclops oithonoides* and *Halectinosoma curticorne* can assimilate dissolved organic nutrients. Fenchel (1972) suggested that deposit feeders feed on living bacteria, but this view is not borne out by our results for *Cyclops*. Another possibility is that deposit feeders use detritus particles for food. Again, Marshall (1973) raised objections to this suggestion, stating that detritus is made up of degraded chlorophyll, pieces of chitinous and siliceous exoskeletons, and other indigestible remains from plants and animals, and is therefore unlikely to be nutritious.

The only alternative that remains is thus dissolved organic substances. Krogh & Berg (1931) found that in the sea dissolved sugars are mostly present as pentosans. However, in the bottom layer there is a possibility that local concentrations of glucose are produced by the foaming action of waves (Baylor & Sutcliffe 1963). Anderson & Stephens (1969) found no significant uptake of either amino acids or glucose by the crustaceans *Artemia salina*, *Tigriopus californicus*, *Limnoria tripunctaya* and *Corophium acherusicum* treated with antibiotics.

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