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ZOOPLANKTON NUTRITION STUDY UNDER CONDITIONS CLOSE TO NATURAL WITH THE USE OF RADIOACTIVE PHOSPHORUS

RECHERCHES SUR LES CONDITIONS D'ALIMENTATION DU ZOOPLANKTON PAR LA METHODE ³²P DANS LES CONDITIONS PROCHES DE CELLES DE LA NATURE.

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Lack of a sensitive method required for in situ study of the nutrition quantitative characteristics (and food balance in particular) concerning zooplankton populations in low-productivity regions was the reason for developing one. For the basis of such a method we selected the property of radioactive phosphorus isotopes of fast and effective embedding into all plankton components (Fyodorov, Sorokin, 1975). The time for plankton labelling was defined by attaining the isotopic balance between the organism and the environment which was normally established after 8-18 hours following the introduction of ³²P or ³³P in the form of orthophosphoric acid without the isotope carrier. Because of the different degree of mineral phosphorus accumulation by microplankton (bacterio-, phyto-, and microzooplankton) and zooplankton, the latter two were decomposed into these components using milling screens with various meshes. To establish the role of individual feed components in nutrition, the animals were given labelled micro- or zooplankton obtained after fractionation of natural plankton and placed in separate aquariums. Natural ratio and concentration of micro- and zooplankton were achieved by combination selection of one labelled food component with other plankton fractions. Feed consumption rate in hydrobionts was determined according to the ³²-³³P label accumulation in their bodies and specific radioactivity of micro- and zooplankton. Stable phosphorus content in the same plankton fraction and bodies of the examined animals was measured by the dry burning method (Lyutsarev, Mirkina, 1975).

Duration of food digestion was determined by the animals' radioactive feces excretion rate. For this purpose individual species, whose intestines were filled with labelled food, were placed in a series of flow-through aquariums (Fig.1) with sea water, natural plankton, additions of stable phosphorus in the form of Na₃HPO₄ (60-120 µgP.l⁻¹) and antibiotics (penicillin, 2.10⁵ a.µ.l⁻¹; streptomycin 0.5 mg.l⁻¹) and rinsed at the rate of 0.5 l.h⁻¹. Additions of the stable phosphorus and antibiotics combined with the sea-water flow reduced totally penetration into microplankton of the radioactive label excreted by animals during the experiment. The feces carried away from aquariums by water were depositing on a funnel with filters (mesh diameter 2.5 µm). Filters were replaced every 15 min., enabling us to trace the dynamics of feces excretion. The end of radioactive feces excretion corresponded to termination of the entrapped labelled food digestion.

In hydrobionts, the food balance was determined in short-term experiments according to the sum of the constituents: the substance accumulated in the body, mineral phosphorus liberated during respiration, feces, and discharge in the form of dissolved organic phosphorus. The amounts of individual elements of the balance were estimated by the values of their radioactivities and specific activity of the feed. In these experiments, the animals were initially kept in vessels with labelled feed until their intestines were filled up, then rinsed from the radioactive agent, and placed into the same flow-through system that was used for estimation of the food digestion duration. The Lugol's solution was added to the water coming from the flow-through system and membrane filters on which the feces were depositing. The solution was fixing the microplankton and ultimately preventing its accumulation and transformation of radioactive metabolic products of the animals under study.

The individual balance elements were determined following the procedure given below. The radioactive mineral phosphorus discharged by animals during respiration was deposited by a magnesia mixture added with NH_4OH in a 200 ml sample with total volume of 1-1.5 l. Then the residue was run through a 1.5 μm -mesh membrane filter (Practical guide on radiochemistry). The dissolved radioactive organic phosphorus was estimated in the filtrate after depositing of the mineral phosphorus. The organic substance was destroyed by ammonium persulphate (Sapozhnikov, 1978) with 120 $\mu\text{g P.l}^{-1}$ then added an mineral phosphorus deposited.

To assess the representativeness of the procedure, the experiments on estimation of individual qualitative copepod feeding characteristics, and the results thus obtained were correlated with the data from literature. The analysis of results obtained by various methods showed their sufficient agreement.

On the basis of the accomplished experiments, we suggest the following experimental pattern employing radioactive phosphorus isotopes in order to investigate qualitative characteristics of zooplanktonic organisms' feeding under conditions close to natural (Fig.2).

Owing to the high sensitivity of the method, the substance transfer rate along the initial links of the nutritional chain may be estimated using natural populations of organisms without violation of the producer-consumer ratios anywhere in the ocean including low-production regions. The opportunity of experimenting with individual species allows to estimate the selectivity of food, variability of rations, food digestion rate, and the elements of their food balance.

The method described above allows not only to quantitatively determine the consumption and transportation of substance by individual species and populations of zooplankton; it rather opens new horizons for investigation of the zooplanktonic association role in regeneration of mineral phosphorus.

This method was applied in zooplankton feeding study in the subequatorial zone of the Indian Ocean. Particular attention was concentrated on copepods, since the share of this group amounted to 50 per cent of the total seston (Piontkovsky *et al.*, 1985).

The investigated area was characterized by heterogeneous quantitative distribution of microplankton. Phosphorus concentration in layers with increased chlorophyll "a" content varied from 0.181 to 1.123 $\mu\text{g P.l}^{-1}$ (Fig.3).

Copepods *Temora discaudata*, *Undinula darwinii*, and *Pleuromamma gracilis*, *P. piseki* showed no specific character during digestion of natural microplankton

whose concentration, in our experiments, totalled 0.445, 0.450-1.123, and 0.227-0.407 $\mu\text{g P.l}^{-1}$, respectively. Depending on the composition of the consumed food, the copepods exhibited unequal character of fecal discharge. For example, all copepods, earlier consuming common microplankton 33p, showed two maximums in fecal discharge: the first, being characterized, as a rule, by higher radioactivity and occurring after 0.5-1.0 h, and the second maximum, occurring after 2-2.5 h following the transfer of the animals from labelled feed to the unlabelled one (Fig.4 a-c). It is likely that the first radioactivity maximum of feces coincides with the flow of the vegetable feed, while the second one indicates the animal feed. This assumption is proved by direct observations made by T.S. Petipa (1985) who examined the digestion process in copepods. The author has established that in mixed feeding, a copepod's intestine was, first of all, forming fecal clots composed of undigested vegetable residues which were excreted in the first place. Animal food was longer retained in the middle part of intestine and excreted much later as compared to the vegetable residues. When consuming small-size fractions of natural microplankton (less than 0.064 mm), *U. darwinii* displayed a different feces excretion pattern (Fig.4): the entire fecal mass was excreted within the first 0.5-1.0 h following the transfer of the animals to unlabelled feed. Therefore, we may assume that which the natural (mixed) microplankton concentration of 0.227-1.123 $\mu\text{g P.l}^{-1}$, calanids under study showed the passing time to vegetable food along intestine of 0.75-1.5, and that of animal, 2-3 hours.

The study of the natural microplankton consumption by copepods revealed that this value varied considerably totalling 0.04-11.9.10⁻⁴ $\mu\text{g P.specimen}^{-1}.\text{h}^{-1}$ or 0.01-0.33 per cent of the body mass. Maximum relative rations were traced in small-size calanids *Acrocalanus monachus* and *Clausocalanus furcatus*, while minimum ones were observed in large-size copepods *Pleuromamma abdominalis* and *Eucalanus* sp. (Table 1).

A certain relation was found between the nutrition intensity of crustaceans and microplankton concentration. For example, the rate of its consumption in *U. darwinii* inhabiting within a rather wide range of microplankton was increasing from 0.2 to 0.7.10⁻⁴ $\mu\text{g P.specimen}^{-1}.\text{h}^{-1}$ as the feed concentration grew from 0.2 to 1.1 $\mu\text{g P.l}^{-1}$ (Fig.4).

Experimental results of studies of the nutritional balance in copepods have shown that within the area investigated and at microplankton concentrations of 0.227-1.123 $\mu\text{g P.l}^{-1}$, *T. discaudata*, *U. darwinii*, *P. gracilis*, and *P. piseki* exhibited a distinct specificity in distribution of substance among separate balance elements (Table. 2). All calanids showed to high values of assimilation efficiency, averaging 71 per cent. The greater part of the assimilated substance (79-90 per cent) was accumulated in animal bodies. Undigested component distribution was about the same in *T. discaudata* and *P. gracilis*, *P. piseki*, though different in *U. darwinii*.

Finally, the results obtained have shown that natural microplankton consumption in copepods under study was low, totalling from fractions to several per cent of the body mass daily. Small-size calanids were satisfying their minimum nutritional demands at the expense of natural microplankton by 10-30 per cent, while large-size copepods, by 2- 10 per cent only. Consequently, mature copepods should depend greatly on other feed sources, the major one being, probably, the animal food.

Another argument speaking in favour of this assumption are the experimental results obtained from the Black Sea *Pontella mediterranea* showing that 95 per cent of their ration consisted of animal food. Here, the natural microplankton

consumption rates in the Black Sea and Indian ocean pontellids were almost alike amounting to 1.5-2.0 per cent of body mass daily. The share of the animal food in the ration of the Indian ocean pontellids may appear the same as in the case with the case with the Black Sea species. This idea is indirectly justified by close concentrations of small-size zooplankton and suspended organic phosphorus in aquatoria under study.

The amounts of microplankton eaten out by mature copepods were estimated on the basis of quantitative data on nutrition and distribution of their population (Piontkovsky *et al*, 1985). Such estimation was made for two layers : the surface layer and the layer with maximum amounts of chlorophyll "a" and microzooplankton. The calculations have proved that microplankton consumption values of this zooplanktonic group dit not exceed 0.5 per cent of its biomass in the above-mentioned layers, or 0.1 per cent of microplankton organophosphorus compounds' synthesis.

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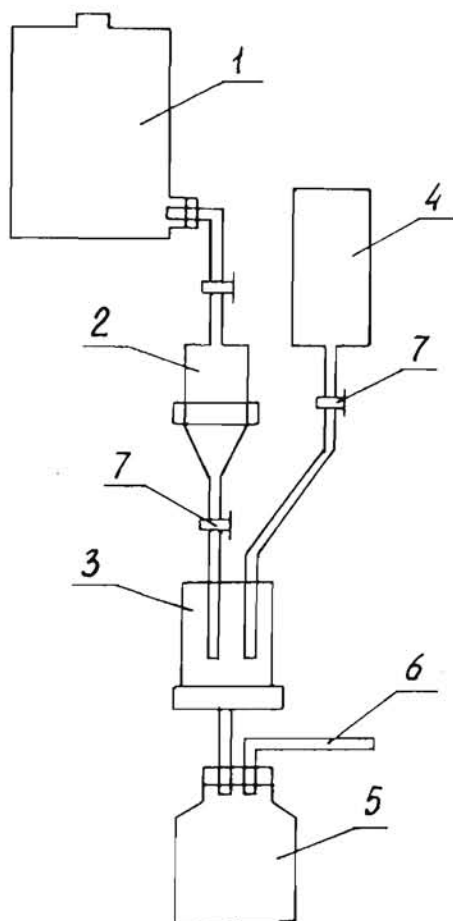


Figure 1 - Diagram of an element of flow-through system for determination of food digestion rate and nutritional balance in zooplankton: 1-vessel with natural sea water ; 2-flow-through aquarium with labelled animals ; 3-funnel with filter for collection of feces ; 4-vessel with Lugol's solution ; 5- reservoir for collection of liquid products of animal metabolism ; 6-vacuum pump ; 7-screw clamps. Complete diagram may consist of a number of elements.

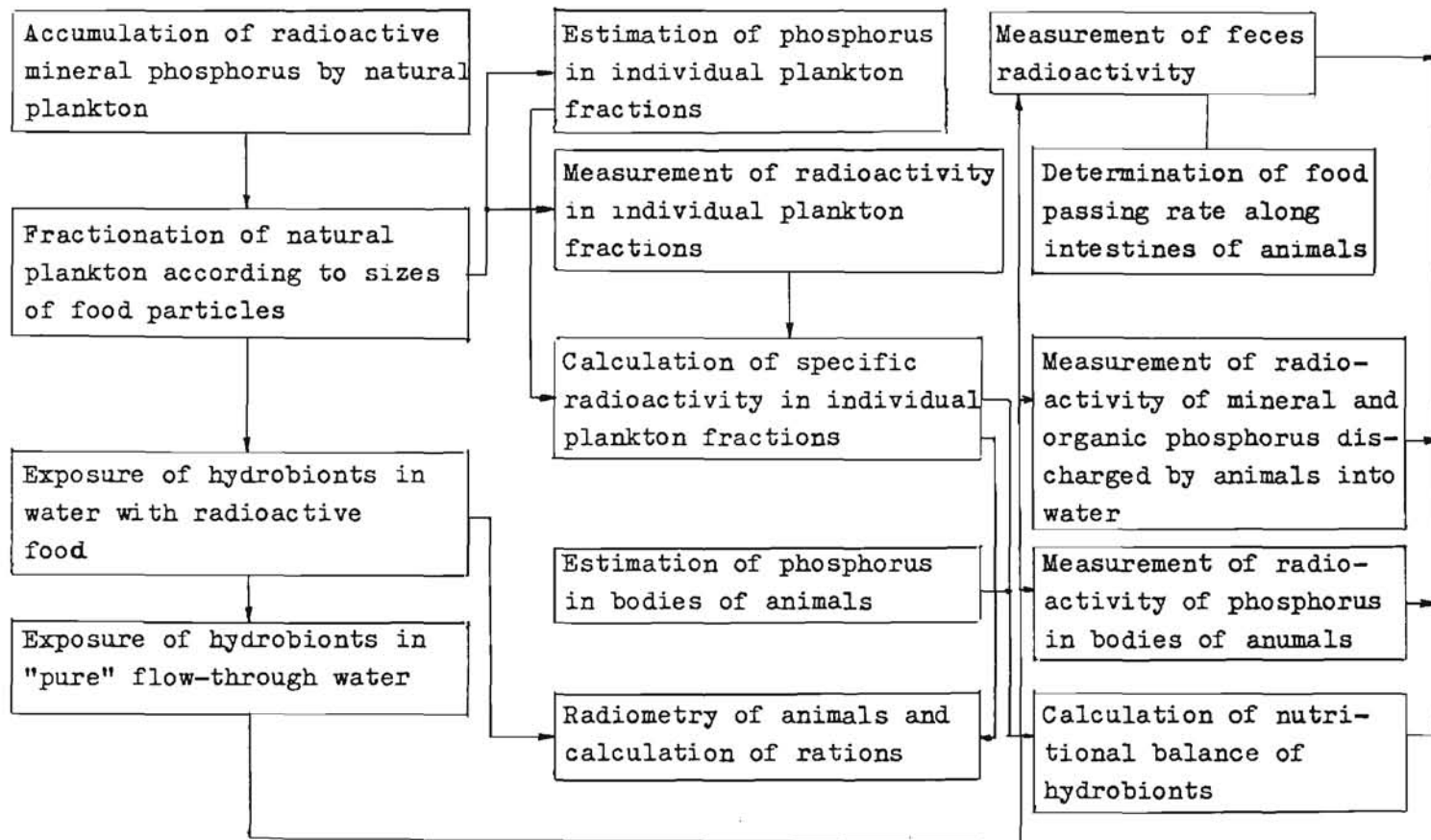


Figure 2 - Experimental set up for studies of nutrition intensity and nutritional balance in hydrobionts under conditions close to natural.

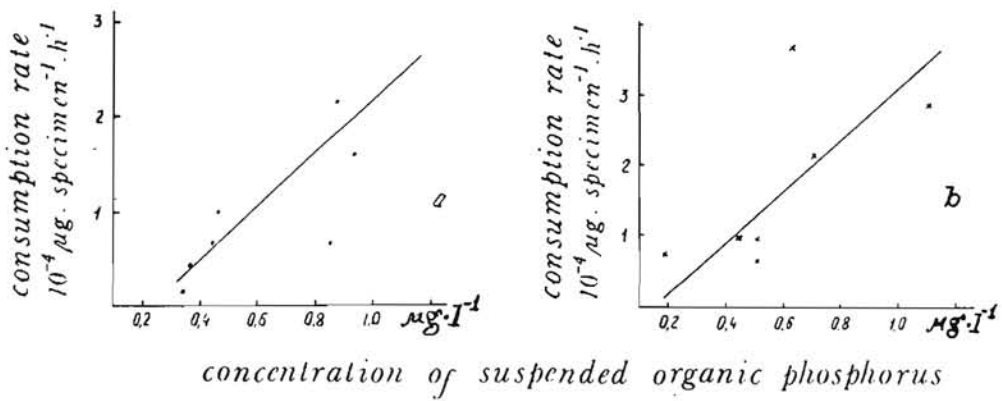


Figure 3 - Food consumption rate versus microplankton concentration in *U. darwinii* (a-night, b-day).

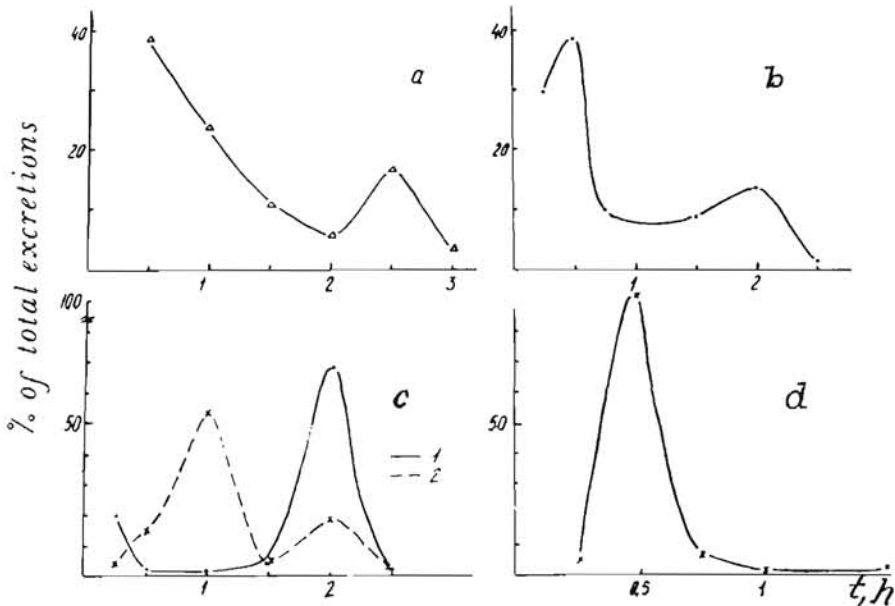


Figure 4 - Fecal discharge dynamics in copepods during natural microplankton consumption (a-*T. discaudata*, b-*U. darwinii*, c-*P. gracilis*, *P. piseki*) and consumption of its small-size fraction (d-*U. darwinii*).

Species	Time	No. of experiments	Average No. of animals in 1 l	Microplankton concentration, $\mu\text{g P.l}^{-1}$	Copepod body mass, $\mu\text{g P. specimen}^{-1}$	Microplankton consumption, hour^{-1}	
						$10^{-4} \mu\text{g P. specimen}^{-1}$	Per cent of body mass
Acrocalanus monachus	day	2	9	0.182-1.123	0.090	0.5-2.4	0.05-0.27
	night	2	7	0.206-0.342		0.6-1.6	0.07-0.18
Calanus minor	night	2	8	0.342-0.943	0.090	0.2	0.03
Clausocalanus furcatus	day	3	12	0.342-1.123	0.092	0.3-3.0	0.04-0.33
Pleuromamma gracilis, P. piseki	day	3	11	0.711-1.123	0.124	0.3-1.2	0.03-0.12
	night	4	7	0.369-0.943		0.2-0.9	0.02-0.09
Undinula darwini	day	6	11	0.182-1.123	0.287	0.2-3.7	0.01-0.11
	night	7	8	0.342-0.943		0.2-1.6	0.01-0.07
Temora discaudata	day	2	10	0.182-0.445	0.301	0.6-4.1	0.02-0.13
	night	2	9	0.219		1.4	0.04
Pontella sp.	day	7	8	0.182-0.644	0.515	0.4-3.8	< 0.01-0.08
Candacia sp.	day	2	8	0.414-0.515	0.776	0.04-0.5	< 0.01-0.01
Euchaeta marina	day	4	4	0.182-1.123	1.020	0.4-5.4	< 0.01-0.05
	night	3	7	0.337-0.734		0.5-1.0	< 0.01-0.01
Pleuromamma abdominalis	day	3	6	0.306-0.890	1.512	0.2-1.6	< 0.01-0.01
	night	4	8	0.337-0.943		0.2-0.7	< 0.01
Eucalanus sp.	day	1	11	0.249	3.400	2.1	0.01
	night	2	5	0.414-0.585		4.0-11.9	0.01-0.04

TABLE I - THE RATE OF NATURAL MICROPLANKTON CONSUMPTION BY MASS SPECIES OF COPEPODS

Species	Microplankton concentration, $\mu\text{g P}\cdot\text{l}^{-1}$	Copepod body mass, $\mu\text{g P}\cdot\text{specimen}^{-1}$	C^P_d	R^P_c	F^P_s	F^P_d	C^P_d	F^P_s	a	Microplankton consumption, hour ⁻¹	
			10^{-4}	$\mu\text{g P}\cdot\text{specimen}^{-1}\cdot\text{h}^{-1}$	$\frac{C^P_d}{R^P_c}$	$\frac{F^P_s}{F^P_d}$	%	%		%	$10^{-4}\mu\text{g P}\cdot\text{specimen}^{-1}$
<i>Temora discaudata</i>	0.445	0.301	1.24	0.12	0.07	0.18	79	39	82	1.61	0.05
<i>Undinula darwinii</i>	0.450	0.287	0.41	0.11	0.10	0.04	79	69	78	0.66	0.02
	0.874 ^x	0.287	0.48	0.01	0.09	0.11	98	45	70	0.69	0.02
	0.511	0.287	0.52	0.02	0.36	0.06	96	87	58	0.94	0.03
	1.123	0.287	2.95	0.50	0.33	0.10	86	90	87	3.88	0.13
<i>Pleuromamma gracilis</i> , <i>P. piseki</i>	0.227	0.124	0.04	0.01	0.01	0.01	80	50	74	0.07	0.01
	0.407	0.124	0.08	0.02	0.01	0.02	80	35	67	0.13	0.01

TABLE II - NUTRITIONAL BALANCE COMPONENT INTERRELATIONS IN COPEPODS (a-T. DISCAUDATA, b-U. DARWINII, c-P. GRACILIS, P. PISEKI ; 1-SHARE OF SUBSTANCE ACCUMULATED IN BODY ; 2-CONSUMPTION FOR RESPIRATION ; 3-SOLID EXCRETIONS ; 4-LIQUID DISCHARGE (PER CENT OF RATION)