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Some Problems Relating to Phosphate Regeneration and Oxygen Consumption in Sea Water
—Phosphate Regenerated by Zooplankton During Excessive Grazing—

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Summary

The rate and the extent of phosphate regeneration and oxygen consumption accompanying the biochemical decomposition of green faecal pellets which herbivorous plankton produce have been investigated in order to obtain the information related to problems of the decomposition of organic matter in sea water.

The rate and the extent of phosphate regeneration and of oxygen consumption increased with rising temperature. The ratio of phosphate regeneration to total phosphorus was little. Between the regenerated phosphate and the consumed oxygen there was a good correlation. During the decomposing stage the relative ratio of oxygen consumption to regeneration of one unit of phosphate is high, and during the last stage it is low.

The studies and estimation of primary production in the ocean were employed in various ways by several groups of researchers up to the present. The primary production, however, affected the following factors: nutrients, light, temperature, oceanic minor-nutrients, and grazing by marine animals (mainly, zooplankton). Among other things, the replenishment of nutrients plays an essential role in the maintenance of primary production. Concerning the origin of replenishment of plant nutrients, it is subdivided into two processes on the whole: the physical processes in the ocean (advection, turbulent exchange) and the remineralization as a result of the biochemical decomposition of organic matter in sea water. The rate and the extent of nutrient regeneration from organic matter decomposing in sea water regulates, in particular, the prosperity and decay of primary production.

The nutrient uptake from sea water by diatom takes place generally in the euphotic zone in the ocean. Subsequently it returns the nutrient to the water by the conversion of organic matter into inorganic compounds both in and below the euphotic zone, following the biochemical decomposition of the diatom cells and the excrement voided by marine animals preying upon the diatom and etc. So far, among the regeneration processes of nutrients attention has been given to the release of significant quantities of nutrients from zooplankton during excessive grazing. On the other hand, the dissolved oxygen in the ocean is consumed owing to the respiration and biochemical decomposition of organic matter such as the excrement of zooplankton.

In this present paper authors have dealt with, *in vitro*, the rate and the extent of phosphate regeneration from the excrement voided by zooplankton preying diatom, at various temperatures (10°C, 20°C, 30°C), and also have obtained the relation between the regenerated phosphate and the consumed oxygen. At the same time, the authors discussed some problems related to phosphate regeneration and the oxygen consumption in sea water, in contrast to present and previous observations as described in this issue.

1. Experimental procedure

The plankton diatom *Skeletonema costatum* (GREVILLE) CLEVE was chosen as a food for the zooplankton, and as materials of zooplankton, *Artemia salina* LEACH was used in this experiment. The procedure for the culture of *Skeletonema costatum* has been shown in detail previously by Motohashi and Matsudaira (1, 2).

After the diatom *Skeletonema* absorbed completely the inorganic phosphate in the culture medium, the starved young *Artemia salina* (ca. 100 individuals) before spawning were added to the culture. The diatom *Skeletonema* was completely consumed within three days after the *Artemia* were added, but still for the purpose of obtaining the green faecal pellets which the *Artemia* void, it was placed in a room thermostated at 20°C temperature for five days. Before the begining of this experiment, *Artemia salina* were gathered by net as a sample of phytoplankton, and the remained mother sample was bubbled with oxygen gas.

The dissolved oxygen, total phosphorus, particulate phosphorus, total dissolved phosphorus, and inorganic phosphate of the first two bottles and the last two were determined at once, these being used to show initial concentration of the subsample in oxygen bottles were dispenced from the mother sample by a syphon. The remaining oxygen bottles of subsamples were placed in separate rooms thermostated at 10°C, 20°C, and 30°C temperature, covered with a sheet of black polyethylen.

After the system was darkening, the subsample in oxygen bottles were periodically withdrawn and analyzed for dissolved oxygen and inorganic phosphate, in duplicate. Analysis for inorganic phosphate was made with filtered water, and analyzed colorimetrically according to the method described by Murphy and Riley (3). The dissolved oxygen was determined by the Winkler method described by Thompson and Robinson (4). The detailed analytical procedures of other phosphorus fractions are described in this issue by Motohashi and Matsudaira (2).

2. Discussion of results

The results are given in Table 1, Fig. 1, 2, and 3.

(1) *Phosphate regeneration*

The initial concentrations of phosphorus fractions of subsample in the oxygen bottles which were dispenced from the mother sample by a syphon already contained 33.5 μg inorganic phosphate, 15.9 μg dissolved organic phosphorus, 64.3 μg particulate phosphorus, and 113.7 μg total phosphorus, per liter. The initial concentration of dissolved organic phosphorus in phosphorus fractions may be ascribed to Hoffman's observations (5), in which approximately one third of total phosphorus is released into the water in a dissolved organic form immediately on the death of the diatom. The initial remarkably high concentration of inorganic phosphate is due to rapid liberation from the excrement voided by the zooplankton during the eight days before the system was set out. It is generally known that the inorganic phosphate from zooplankton during excessive grazing tends to liberate rapidly as compared with the biochemical decomposition of diatom cells. Gardiner (6) observed that during the feeding of zooplankton, the inorganic phosphate increased in the water. It appears that in the green faecal pellets which herbivorus plankton produce, some of the organic phosphorus is in a labile state and phosphatases can proceeds rapidly and directly.

In this present experiment, however, the rate and the extent of phosphate regeneration from excrement voided by *Artemia salina* grazing upon diatom

TABLE 1. *Changes in the concentration of inorganic phosphate (Inorg. PO_4-P; expressed in $\mu g/L$) and dissolved oxygen (O_2; expressed in ml/L) at various temperatures.*

Time, Days	30°C temp.		Time, Days	20°C temp.		Time, Days	10°C temp.	
	O_2	Inorg. PO_4-P		O_2	Inorg. PO_4-P		O_2	Inorg. PO_4-P
0	10.94	33.5	0	10.94	33.5	0	10.94	33.5
2	10.29	38.8	4	10.61	34.5	5	10.39	34.6
5	9.62	39.9	9	9.98	38.1	15	9.71	37.5
12	7.45	44.3	17	9.56	39.2	25	9.13	40.7
19	7.26	46.0	25	9.28	39.5	41	9.00	39.2
29	6.73	49.2	35	8.59	41.4	62	8.76	40.9
40	6.29	51.1	47	8.31	42.3	93	8.11	41.6
52	6.00	55.4	64	8.16	43.0	123	7.92	45.4
64	6.07	53.9	83	7.16	45.2	—	—	—
83	5.77	60.4	105	6.86	48.2	—	—	—

Total phosphorus ; 113.7 $\mu g/L$

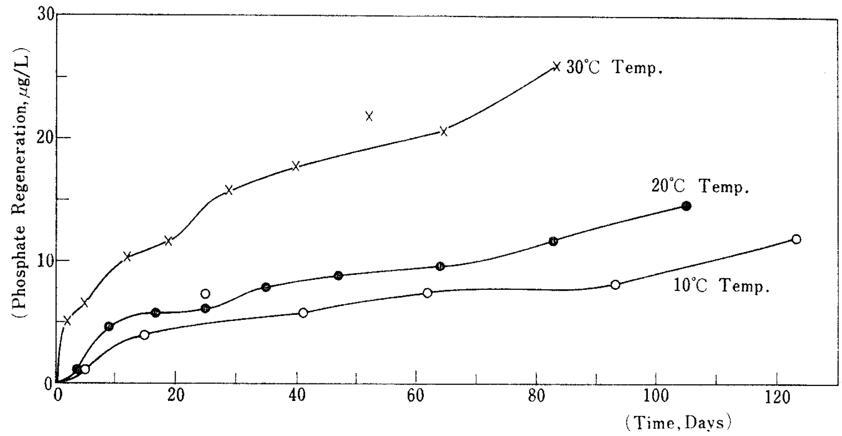

FIG. 1 Regeneration of inorganic phosphate from the excrement voided by *Artemia salina* under various temperatures.

Skeletonema is that during eighty-three days it amounted to 26.9 μg per liter at 30°C temperature, in which the ratio of inorganic phosphate to total phosphorus is only ca. 24 per cent (Fig. 1, Table 1). Meanwhile, at 20°C and 10°C temperature the regenerated phosphate after darkening was no more than the concentrations of 14.7 μg per liter during a hundred five days and of 11.9 μg per liter during a hundred twenty-three days, respectively. Also, the rates of phosphate regeneration at both temperatures were very slow (Fig. 1, Table 1). Thus it is concluded that the rate and the extent of phosphate regeneration accompanying biochemical decomposition of excrement voided by zooplankton increase with rising temperature.

Although it was pointed out previously by Motohashi and Matsudaira (2) that during the first decomposing processes of diatom cells an exponenetial phase appears at various temperatures, its appearance was not observed in this experiment. And also results corresponding to Gardiner's observation could not be obtained, in contrast with the one about the diatom cells as described in this issue. For these causes it may be infered that an exchangeable fraction of the diatom cells in green faecal pellets voided by *Artemia* had been oxidized already before the system was set out, or that the *Artemia* excreted a great quantity of pseudofaeces caused by excessive grazing. Goldberg et al. (7) pointed out that the content of phosphate in the diatom cell is subdivided into two fractions; namely, a fraction of exchangeable organic phosphate influenced more by oxidation and another fraction of minimum fraction hardly affected by oxidation. It is, on the other hand, known that zooplankton voids undigested pseudofaeces during excessive grazing.

So far, the release of significant quanties of phosphate from the zooplankton during excessive grazing was called to attention as an important process affecting the conversion of organic matter into inorganic compounds. Considering the above results, however, it is not necessarily confirmed. Therefore, we should keep in mind that the rate of release of phosphate by zooplankton during excessive grazing may be controlled by the amount of green faecal pellets that the herbivorous plankton produce, or by the amount of undigested pseudofaeces that is excreted because of the excessive grazing of the zooplankton. At the same time, the relation between the standing crop of zooplankton and diatom, and its amount of diatom grazed by zooplankton should be taken account into the basic problem of reproduction that is related to the nutrient regeneration from organic matter decomposing in sea water.

(2). *Oxygen consumption*

It has been shown previously by Motohashi and Matsudaira (2) that the rate and the extent of oxygen consumption by the diatom decomposing in stored sea water is regulated by the amount of oxygen-consuming organic matter, and that the relation between the oxygen consumption and the phosphate regeneration depends on the relative amount of diatom population to the dissolved oxygen content of sea water. Based on the above observation, rapid oxygen consumption is expected, if the organic phosphate from the excrement of zooplankton during excessive grazing tends to a more rapid liberation as compared with that of the breakdown of diatom.

The rate of oxygen consumption accompanying biochemical decomposition of the excrement voided by zooplankton is shown in Fig. 2. At 30°C temperature the dissolved oxygen is consumed exponentially in this experiment for the first fifty days. During the first ten days the extent of oxygen consumption amounted to more than 3.00 ml per liter. It is not all but consumed during the last thirty days. Meanwhile, the dissolved oxygen at 10°C and 20°C temperature is consumed at the same rate during the first thirty days and its consumption amounts to ca. 2 ml per liter. After this, at 20°C the dissolved oxygen consumed slightly more as compared with the one at 10°C, but the oxygen consumption in both temperature is generally slow. The time taken for the consumption to 3.00 ml per liter of dissolved oxygen was more than eighty days at 20°C and more than a hundred twenty days at 10°C temperature (Fig. 2).

From the above results, the expected rapid oxygen consumption could not be observed, and rather a rapid consumption was observed on the respiration and biochemical decomposition of the diatom cell in previous experiment (Motohashi and Matsudaira, 2). This is attributable to the good correlation between the consumed oxygen and the regenerated phosphate from organic matter decomposing in stored sea water, in which the rate and the extent of oxygen consumption is regulated by the amount oxygen-consuming organic matter. In this case, however, the character of the organic matter was green faecal pellets which herbivorous plankton produced, and furthermore there appear pseudofaeces which the Artemia

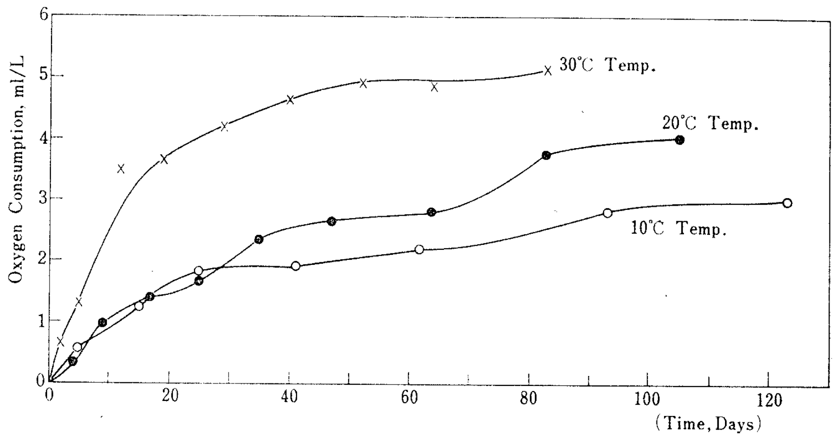

FIG. 2 Oxygen consumption as a result of the biochemical oxidation of the excrement voided by *Artemia salina*.

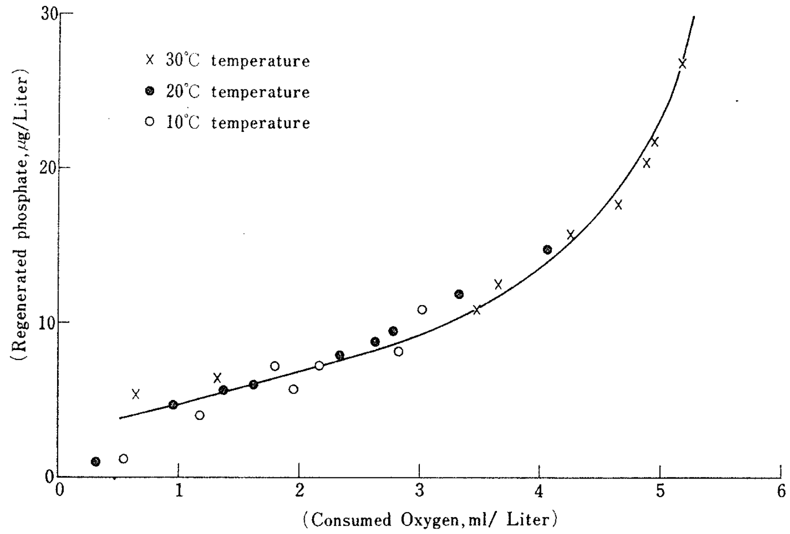

FIG. 3 Relation between the consumed oxygen and the regenerated inorganic phosphate.

voided because of excessive grazing. Concerning the pseudofaeces voided by the zoolplankton during excessive grazing, Watanabe* suggested that they are not easily affected by biochemical oxidations. Finally, in this present experiment a slow oxygen consumption may be mainly ascribed to a great quantity of pseudofaeces.

(3). *Phosphate-oxygen relation*

With the intention of obtaining the correlation between the biological activity and the ratios of nutrient composition in the ocean, Fleming (8), Matsudaira et al. (9), Redfield (10), and others had advanced a general idea of AOU (Apparent Oxygen Utiliztion), on the assumption that at one time every parcel of water was fully saturated with oxygen. This concept of AOU does not take into account the

* Personal communication

physical structure in the ocean, nor the complexity of the uptaking processes and the remineralizing processes of nutient by biological activity. In addition to the observations obtained previously about phosphate regeneration from diatom decomposing in stored sea water, the relation betweeen the regenerated phosphate and the consumed oxygen accompanying biochemical decomposition of excrement that herbivorous plankton produced were examined.

The relation between the regenerated phosphate and the consumed oxygen accompanying biochemical decomposition of the excrement voided by zooplankton during excessive grazing is exponential like that of diatom (Fig. 1). Namely, during the first decomposing stage the relative ratios of oxygen consumption to regeneration of one unit of phosphate is high, and during the last stage it is low. In contrast to previous observations, here, the exponential curvature differs. This difference which had been already discussed by Motohashi and Matsudaira (2) may depend on the relative amount of easily oxidable organic matter and the dissolved oxygen content of the water during the decomposition.

According to the concept of AOU, on the other hand, the phosphate-oxygen relation is not exponential but linear, because of a supporting and useful assumption in chemical oceanography, that the amount of inorganic phosphate derived from oxidative organic phosphorus is directly proportional to the loss in the oxygen if a parcel of water would be carried down from the sea surface where presumably it was fully saturated with oxygen. Also 1 μg A. of inorganic phosphate is produced at the expense of 276 μg A. of oxygen on the premise that the ratios of nutrient regneration are the same as the ratios of average plankton composition.

However, there is a fact found from the results of present and previous observations that the regeneration of phosphate is not proportional to oxygen consumption, but it has a logarithmic relation. In order to obtain a reasonable correlation of phosphate and oxygen, therefore, we should investigate the fundamental problems related to the movement of water masses in the ocean, in addition to the relation between the amount of oxygen-consuming organic matter and the dissolved oxygen content in sea water. Because the dissolved oxygen content in sea water is altered by not only biochemical action, but also the following physical factors such as thermal structure, wind mixing, changes in barometric pressure, relative humidity, advection and diffusion across sea water.

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