

УДК 574.5

Excretion by Benthic Invertebrates as Important Source of Phosphorus in Oligotrophic Ecosystem (Lake Krivoe, Northern Russia)

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Received 02.03.2016, received in revised form 11.06.2017, accepted 29.11.2017

*We studied contribution of benthic invertebrates to the dissolved phosphates flux from the bottom sediments to the water in an oligotrophic lake (Lake Krivoe, near the White Sea biological station “Kartesh”, Northern European Russia) in 2009–2013 with the aim to quantify specific (among and within taxa), spatial (littoral and deep sites) and seasonal variability. The excretion rates (P_{exc}) of soluble reactive phosphorus (SRP) were estimated in experiment with dominating taxa of benthic invertebrates (amphipods, oligochaetes, mollusks, and chironomids), freshly collected from the lake. Mass-specific P excretion rate was calculated as P_{exc} divided by animal dry mass and further used for calculations of benthic P efflux rates. Also, in other experimental series with and without animals (amphipods) we measured extracellular alkaline phosphatase activity. The results showed that the SRP excretion rates varied significantly between taxa and between different-sized individuals within the same taxa (the SRP excretion rate elevates with an increase in individual weight). Notable spatial and seasonal differences in biomass of benthic animals and phosphorus released by them in the lake were found. The calculated phosphorus efflux by excretory activity of benthic animals was more intensive in littoral sites (up to $14.4 \mu\text{mol m}^{-2}\text{d}^{-1}$ in July), that 7 times greater than maximum efflux in deep areas ($2.2 \mu\text{mol m}^{-2}\text{d}^{-1}$). The mobile burrowing animals (the amphipods *Gammarus lacustris*, *Monoporeia affinis*, and *Gammaracanthus loricatus*) are able to move from the bottom sediments to water and transfer the nutrient; they contributed 80 % of P flux caused by benthic excretion in the lake and affected the activity of the extracellular alkaline phosphatase, increasing its level in water*

3-8 times relative to that in the control (without animals). The high phosphatase activity may increase phosphorus bioavailability by catalyzing hydrolysis of organic phosphorus, and further stimulate primary producers.

Keywords: phosphorus cycle, excretion, zoobenthos, amphipods, phosphatase activity.

Citation: Berezina N.A., Maximov A.A., Umnova L.P., Cao X., Arakelova E.S., Zhou Y., Golubkov S.M. Excretion by benthic invertebrates as important source of phosphorus in oligotrophic ecosystem (Lake Krivoe, Northern Russia). J. Sib. Fed. Univ. Biol., 2017, 10(4), 485-501. DOI: 10.17516/1997-1389-0046.

Экскреция бентосных беспозвоночных как важный источник фосфора в экосистеме олиготрофного озера

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Работа посвящена количественной оценке вклада бентосных беспозвоночных в поток растворенных фосфатов из донных отложений в воду в олиготрофном озере (оз. Кривое возле Беломорской биологической биостанции «Картеш», север европейской части России) в 2009–2013 гг., которая проводилась с учетом специфических (межвидовых, внутривидовых), пространственных (литораль, профундаль) и сезонных изменений. Удельную по массе тела скорость экскреции фосфатов ($P-PO_4^{3-}$, растворенная форма) у доминантных по биомассе бентосных беспозвоночных (амфипод, олигохет, моллюсков и хирономид), собранных в озере, измеряли экспериментально после 4 ч экспозиции в воде с известной начальной концентрацией фосфатов и, совмещая с биомассой таксонов в бентали, использовали для расчета общего потока фосфатов. Кроме того, в других экспериментальных сериях с животными и без животных (амфиподы) измеряли активность внеклеточной щелочной фосфатазы в воде. Результаты показали, что уровни экскреции фосфатов значительно различаются между отдельными таксонами и между индивидуумами разной массы в пределах одних и тех же таксонов (скорость экскреции повышается с увеличением индивидуальной массы животных). Обнаружены большие пространственные и сезонные различия в биомассе бентосных животных и количестве фосфора, выделяемого ими в озере. Вычисленный поток фосфора за счет экскреторной активности бентоса был более интенсивным в литорали, достигая $14.4 \mu\text{моль м}^{-2} \text{сут}^{-1}$, что почти в 7 раз превышало максимальный поток в профундали озера

(2.2 $\mu\text{mol m}^{-2} \text{ сут}^{-1}$). Амфиподы *Gammarus lacustris*, *Monoporeia affinis* и *Gammaracanthus loricatus*, способные активно перемещаться в толще донных отложений и плавать в воде, переносили путем экскреции до 80 % от общего потока фосфатов и влияли на активность внеклеточной щелочной фосфатазы, увеличивая ее активность в 3-8 раз по сравнению с контролем (без животных). Высокая активность фосфатазы может увеличить биодоступность фосфора, катализируя гидролиз его органических форм, что, в свою очередь, стимулирует рост первичных продуцентов.

Ключевые слова: фосфор, экскреция, зообентос, амфиподы, активность фосфатазы.

Introduction

Being a key element in DNA and a variety of other biomolecules, phosphorus (P) is a vital element for all living organisms (Westheimer, 1987) and is the limiting element for photosynthetic primary production in freshwater ecosystems (Schindler, 1977; Hecky, Kilham, 1988). Lake sediments are known to be a long-term sink for P, and its capacity to retain P affects ecosystem productivity to a large degree. The level of sediment oxygen is important for the burial and recycling of dissolved phosphates, and in oxic sediments, usually relatively P rich, low or even negative fluxes (i.e., uptake of dissolved inorganic phosphorus (DIP) with pore water) are often estimated, indicating efficient trapping mechanisms of DIP (Ingall et al., 1993).

Internal P sources such as P-flux via sediment-water interface by physical, chemical, and biological mechanisms may play important role for producers in lakes with low external load (from catchment areas). At present, the conceptual theory of role of aquatic animals in internal P load in lakes left a lot of unsolved questions, including relationships between efficiency of food assimilation and P regeneration. Non-assimilated phosphorus may be excreted at various proportions, and assimilated phosphorus is also may variably be released with metabolites. Phosphorus used for the somatic growth (containing in benthic

biomass) may later be included in the P cycling through food web interactions.

It was shown that benthic animals can release dissolved phosphates from oxic sediment through their excretory activity (Ji et al., 2011). They can excrete P directly by egestion, releasing particulate phosphorus with feces and dissolved phosphorus with urine. P release rate was found to be a function of the mass of animals' body (Schindler, Eby, 1997; Golubkov, Berezina, 2012) and P content in their food (Vanni, 2002; Griffiths, 2006). Also, P content in the body of aquatic animals correlates positively with P excretion rate (Torress, Vanni, 2006).

Differences in the P excretion rate between diverse benthic taxa determine their function in ecosystem and might be important for understanding of their role in nutrient cycling. For example, the mobile benthic amphipod *Gammarus lacustris* Sars, 1863 released notably more P (Wilhelm et al., 1999) than infaunal burrowing chironomids (Tarvainen et al., 2005).

In addition, quantification of the role of benthic macrofauna as suppliers of dissolved phosphates and their bioavailability for algae and bacteria is necessary. It is considered that aquatic animals release dissolved phosphorus in the form of soluble reactive orthophosphates (SRP), which is almost completely available for algae (Vanni et al., 2001). On the other hand, some studies found that dissolved P fraction was excreted in

organic form by some amphipods and tubificids (Johannes, 1964; Gardner et al., 1981). In this case, direct relationships between P excreted by animals and enzymatic activity of bacteria and algae could be expected.

It is known that bacteria and microalgae produce extracellular phosphatases to hydrolyze organic phosphorus for compensation of ambient available phosphorus deficiency in lake system (Cao et al., 2005). Also, it had been shown that some aquatic animals can affect the activity of the extracellular enzymes of bacteria or algae (Vrba, Machacek, 1994; Luo, Gu, 2016). For example, burrowing mangrove crab impacted the sediment nutrient cycling, thereby accordingly changing enzymatic activity (including acid phosphatase) and number of bacteria (Luo, Gu, 2016).

By present, there is some data on spatial and seasonal variability of dissolved phosphorus release by zoobenthic taxa in eutrophic ecosystems (Devine, Vanni, 2002; Ji et al., 2011, 2015), but there is no any quantitative data on benthic animal's contribution to internal P flux in oligotrophic lakes. Low-populated northern region of Russia is rich in clear-water lakes (more than 60 000 lakes) that are characterized by scarce external load and low production. This paper focuses on study of biological mechanisms of dissolved phosphate release in sediment-water interface in oligotrophic lake. The study was performed in high-latitude freshwater lake, situated in the northern Karelia on White Sea coast (Lake Krivoe). Main purposes include: study of differences in P excreted among and within taxa; evaluation of their contribution to the P flux; quantification of spatial and seasonal variability of benthic biomass and excreted phosphorus in the lake. Furthermore, we verified experimentally idea that excretory activity of benthic animals can influence the extracellular phosphatase activity and, therefore, stimulate growth of producers.

Material and methods

Study lake

Lake Krivoe (66°21' N, 33°38' E) is sub-arctic oligotrophic small lake, located on the shore of Chupa Bay, White Sea near Cape Kartesh, Northern Russia (Fig. 1). This lake is the most typical lake on the Baltic crystal platform (Table 1). Littoral (depth < 3 m) contributed 20 % to the total lake area (0.5 km²). Sediments consisted of finely divided grey silt, clays, mineral and organic matter in central sites, while sand, stones, and relatively coarse materials (leaves, detritus) are typical in littoral zone.

Sampling

Samples of macrozoobenthos were collected from four sites, two deep water (sites 1 and 2) and two shallow-water littoral (sites 3 and 4, Fig. 1) during the season (May-October) of 2009-2013 in order to study the benthic taxonomical composition, abundance, biomass and dominating taxa (> 10 % of the total biomass). Samples were taken with Van Veen grab (0.025 m²) at deep sites and by Mordukhai-Boltovskoj core sampler (0.005 m²) at littoral sites in five and six replicates, respectively. The samples were sieved through a 0.25 mm mesh, rinsed with the clean water, preserved with 10 % formaldehyde and stored in plastic zip-bags before laboratory analysis.

The abundance and biomass of the megabenthic (body length up to 40 mm) mobile amphipod *Gammaracanthus loricatus* (Sabine 1824) (subsp. *aestuariorum*), common at depths of 24–32 m, was determined by trapping and the Petersen's marking method (Ricker, 1975). This species inhabits the deepest part (10 %) of the lake, where traps were hanged. Two hundred marked individuals (M) were released into the lake in the 6x6 m² area within location of this species (St 1). Marks were made by light mutilating on third left coxal plate of each amphipod. Four days after this

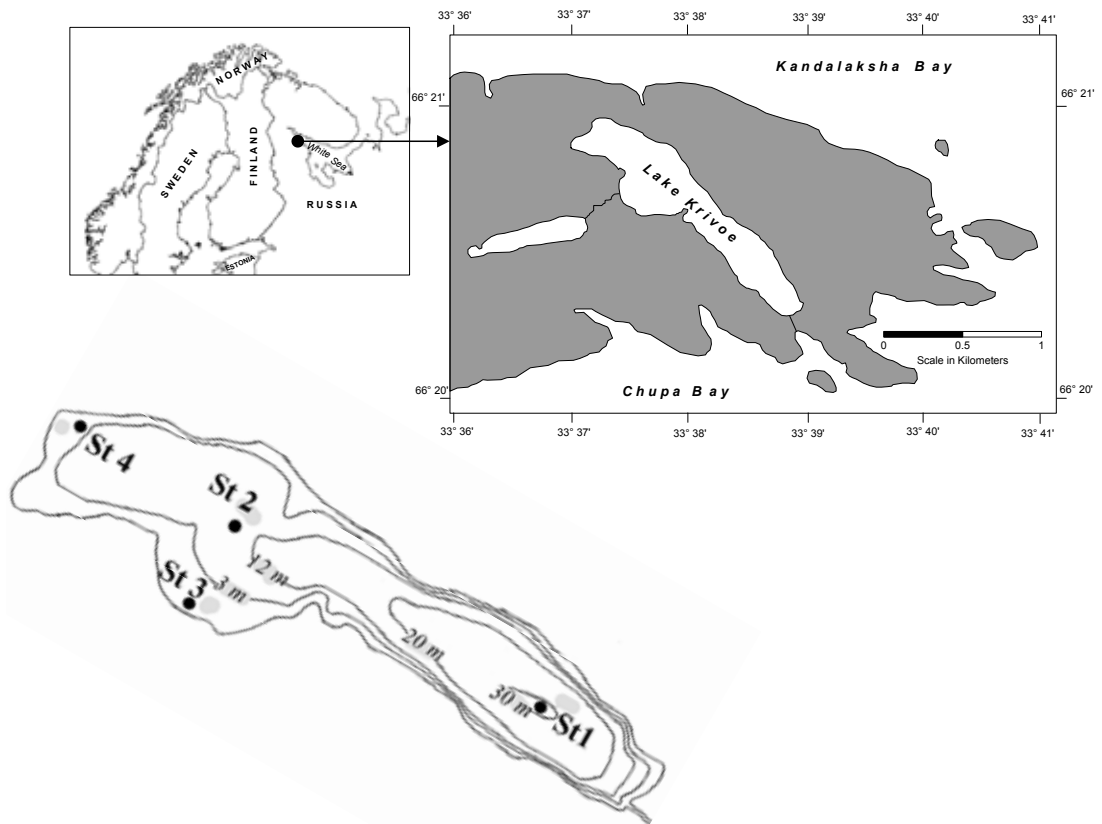


Fig. 1. Location of Lake Krivoe (Kandalaksha Bay coast, White Sea) and bathymetric map showing sampling sites (St. 1-4)

Table 1. Main physical and chemical variables of Lake Krivoe during period of study

Parameters	Variables
Surface area, km ²	0.5
Mean (max) depth, m	12 (32)
Transparency, m	4.5–6.8
Water color, Pt-Co	30
Mineralization, mg L ⁻¹	45–50
Surface (bottom) Oxygen, mg O ₂ L ⁻¹	8.6–12.1 (4.9–11.4)
Surface (bottom) pH	7.4–7.7 (6.8–7.3)
Total phosphorus (TP), mg m ⁻³	8–84
Particulate phosphorus (PP), mg m ⁻³	1.7–6.7
Chl a, mg m ⁻³	0.2–3.0
Primary production rate, mg C m ⁻² d ⁻¹	
Phytoplankton	85–580
Macrophytes	500

release, over-night trapping was performed. The *G. loricatus* population size (N) was estimated taking into account total number of marked (M) and caught (C) animals, and number of marked animals caught in the trap (R, recaptured marks): $N = MC/R$.

In laboratory all animals were picked out the benthic samples under stereoscopic microscope (MBS-10) and sorted into eight taxonomic groups: Oligochaeta, Hirudinea, Gastropoda, Bivalvia, Amphipoda, Chironomidae, aquatic insects and others. The animals were counted and weighed on an electronic balance to the nearest 0.01 mg. Before weighting water was removed from animal body with filter paper (wet weight, the mollusk shells are included). To get dry weight, material was dried in thermostat at $T=50-60$ °C for 96 h to constant weight.

Experiments

Animals of seven dominating taxa were collected at study sites to measure dissolved phosphate excretion rate in laboratory experiments: amphipods (*Gammarus lacustris*, *Monoporeia affinis* (Lindström, 1855), and *Gammaracanthus loricatus* (subsp. *aestuariorum*)), mollusks *Lymnaea stagnalis* (Linnaeus, 1758) and *Sphaerium sueticum*¹ (Clessin in Westerlund 1871), oligochaete *Lumbriculus variegatus* (Müller, 1774), and larvae of the chironomids *Sergentia coracina* (Zetterstedt, 1850). Larvae of aquatic insects (Ephemeroptera, Trichoptera, Plecoptera, Odonata and other insects) are regarded as temporary inhabitants in the lake; high number of species occurred in May-June at generally low biomass (< 5 %) distributed irregularly. Therefore, they were not used in measurements within the frame of this study.

¹ Some authors consider that *Sphaerium sueticum* is a synonym of *S. corneum* Linnaeus 1758 (Vinarski, Kantor, 2016). The taxonomy of genus *Sphaerium* needs further clarification, so individuals from Lake Krivoe, used in our experiments, were identified as *S. sueticum*.

The P_{exc} by some aquatic insects and other species from the lake were provided in (Golubkov, Berezina, 2012).

The excretion rates of dissolved orthophosphates $P-PO_4^{3-}$ (soluble reactive phosphorus, SRP) were estimated by the method described earlier (Devine, Vanni, 2002; Ji et al., 2011). The P excretion rate was measured in each taxon separately, using from 1 to 20 individuals of each species and increasing the number from larger to smaller individuals. The animals were put in 50-120 ml bottles filled with filtered (through a 0.45 μm pore size membrane filter) lake water and were incubated in the dark during four hours at temperatures 20 °C and 10 °C. The control (bottles without animals) was incubated at the same conditions to find changes in the nutrient due to other reasons than animals' activity.

P excretion rate (P_{exc} , $\mu g P h^{-1}$) was calculated as a difference between SRP concentrations in treatment and control divided by experiment duration (4 h). Obtained empirical data on P_{exc} were presented as a power function of individual wet body mass. P_{exc} divided by dry weight (DW, mg) of animals or the mass-specific P excretion rate (P_{exc}/DW , $\eta mol mg^{-1} h^{-1}$) was further used for calculations of benthic P efflux rate. Total P efflux rate ($\mu mol P m^{-2} d^{-1}$) was calculated as a sum of SRP released by each taxon for 24 h multiplied by its biomass in the lake.

The value of the temperature coefficient (Q_{10}) was calculated for each species by the formula: $Q_{10} = (R_2/R_1)^{10/(T_2-T_1)}$, where R_n is the SRP excretion rate at the temperature T_n (°C), using the rates measured at T 10 °C and 20 °C. It characterizes the rate of an increase in P_{exc} for every 10-degree rise in water temperature. Particularly, it shows P_{exc} differences between warm and cold periods. This coefficient was used at calculations of P efflux at different water temperature (seasonal changes).

To measure the alkaline phosphatase activity (APA) we performed 4 h exposition of bottles filled with lake water without and with field-collected 3-15 ind. of the amphipod *G. lacustris*. The experiments were performed as described above but at light and 20 °C.

APA and SRP in water and weight of animals were measured immediately after experiment.

Phosphorus and phosphatase activity measurements

Phosphorus that reacted with ammonium molybdate was considered to be soluble reactive inorganic phosphorus (SRP). SRP in water was estimated using a standard method with photolorimetry (Murphy, Riley, 1962). The total phosphorus (TP) was determined by the method established by Golterman et al. (1978).

Bulk extracellular alkaline phosphatase activity (APA, $\mu\text{mol l}^{-1} \text{h}^{-1}$) in the water samples was determined using a procedure modified from Gage and Gorham (1985) and Boon (1989). APA was determined in unfiltered samples. Triplicate 5 mL water samples were supplied with Tris-HCl buffer (pH 8.5; final concentration 13 mmol L^{-1}), Na_3N (final concentration 5 mmol L^{-1}), and p-nitrophenyl phosphate (final concentration 0.3 mmol L^{-1}), and incubated at 37 °C for 4 h. Absorbency of p-nitrophenol was measured spectrophotometrically at 410 nm in a 1-cm cuvette. Sterilized distilled water was taken as a blank sample.

Statistics

All measured parameters were expressed as mean \pm SE (standard error). Nonparametric Spearman Rank Correlation coefficient served to test relationships between SRP, APA and abundance and biomass of animals. Differences in variables between sites, months were estimated by analysis of variance (ANOVA). The t-test was used to determine if two sets of data were

significantly different from each other. Prior to the analysis, data were tested for homogeneity and Lg (x+1)-transformation was used in the case of heterogeneous variances. Pair-wise comparison of slope and intercept of regression lines was performed.

Results

Lake characteristics

Table 1 summarizes the basic physical and chemical characteristics of the lake. From first studies of this lake in 1960s (Winberg, 1975) it remains oligotrophic ecosystem with low nutrients and primary production of phytoplankton as well as macrophytes due to general hydrological features of lakes in northern region and low anthropogenic impact on its ecosystem. Lake situates in unpopulated region of Russia and only seasonal biological station (with 200 people's annual attendance) acts nearby its shore since 1957. The ice-free period at the Lake lasts from late May-June to October, approximately 140 days; from November to late May it is ice-covered. The surface water temperature reaches 4–5 °C in May–early June, 10–16 °C in June and August-September, and maximum in July (about 20 °C). As a rule, from June to September, a thermocline is recorded at depths of 5–7 m with > 10 °C in the epilimnion and 4–7 °C in deeper water. The bottom waters at deepest part (30 m) keep constantly 4–5 °C and 60–90 % of oxygen.

Total phosphorus (TP) concentration varied from 8 to 84 mg m^{-3} , with maximum in surface waters under ice in April or at the beginning of May (Fig. 2). Also, lake ice (50–60 cm thick) contained 54–76 mg m^{-3} of TP. Particulate phosphorus contributed from 4 to 42 % to the water TP tending to some elevation in hypolimnion. Dissolved orthophosphates ranged 0.1–12.5 mg m^{-3} in June and increased (29–44 mg m^{-3}) in August, with a maximum in the bottom water (Table 2).

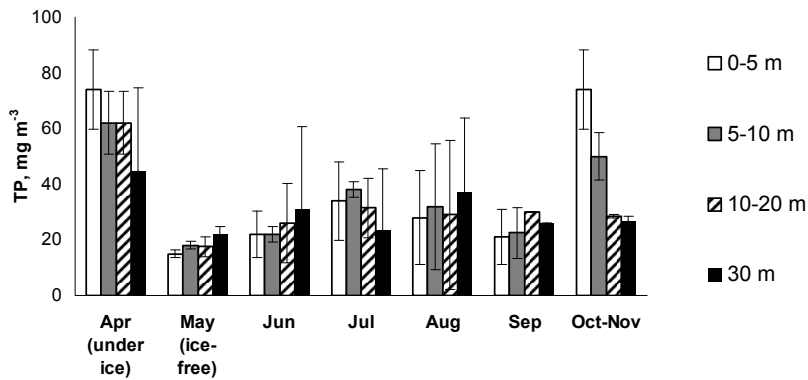


Fig. 2. Seasonal dynamics of the total phosphorus (TP, mg m⁻³) in water column of Lake Krivoe (means ± standard deviations for 2009-2013)

Table 2. Dissolved orthophosphates (PO₄³⁻, mg m⁻³) at various depths of Lake Krivoe during summer months (PO₄³⁻ is equivalent to SRP)

Depth (m)	Month	PO ₄ ³⁻ , mg m ⁻³
Surface (0-5)	June	0.1–10.0
	July	11.6–12.5
	August	29.3
5-10	June	2.6–10.0
	July	12.0
	August	29.0–32.0
10-20	June	5.1–12.5
	July	14.4
	August	31.0–35.0
Bottom (30)	June	10.0
	July	20.0
	August	43.6

Abundance and biomass of zoobenthos in lake

The total biomass of macrozoobenthos varied between 0.2 and 23 g m⁻² (wet weight) achieving maximum in littoral sites (Fig. 3). Six dominating species were distinguished: the amphipods *G. lacustris* and *M. affinis*, the oligochaete *L. variegatus*, the chironomid *S. coracina*, the gastropod *L. stagnalis*, and the bivalve *S. suecicum*. Maximum biomass 22 g·m⁻² at St 3 was recorded in March 2011 and 23 g·m⁻² at

St 4 in May-June 2010 (Fig. 3), mainly on account of high development of the amphipod *G. lacustris* and the bivalve *S. suecicum*.

The megabenthic amphipod *G. loricatus* (body length 22-40 mm and body wet weight 0.187-0.878 g) was common at St 1. Estimated by Petersen's marking method, its abundance and biomass varied in July-September in the ranges of 10-30 ind. m⁻² and 5-25 g m⁻², respectively.

Significant differences in the total biomass were found between littoral and deep sites in

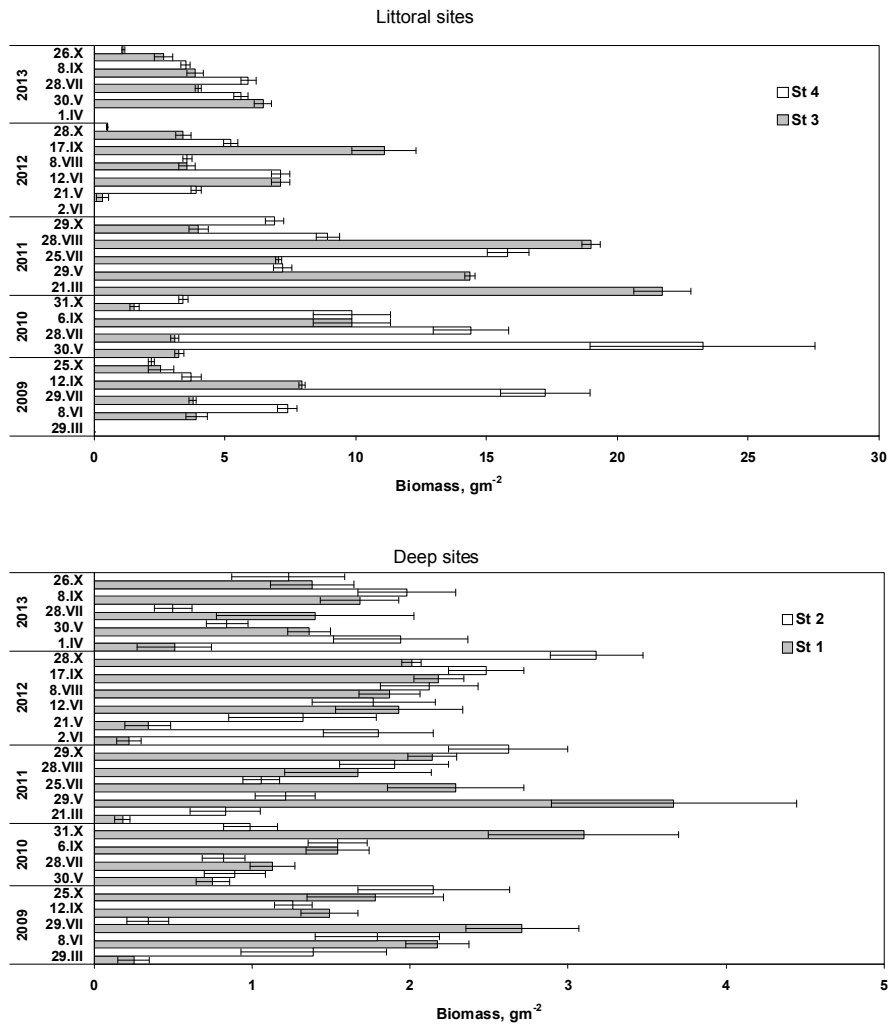


Fig. 3. Seasonal dynamics of the total wet biomass of zoobenthos (mean ± standard error) at four study sites of Lake Krivoe in 2009-2013

Table 3. Average seasonal (March-October) biomass (g m⁻²) and P-efflux (μmol m⁻² d⁻¹) ± SE for whole period of observation (2009-2013) of macrozoobenthos at various sites of Lake Krivoe

Variable	St 1	St 2	St 3	St 4
Biomass	1.59±0.24	1.50±0.18	6.58±1.22	8.08±1.21
P-efflux	1.26±0.20	1.41±0.23	6.50±1.11	7.62±1.75

the lake (ANOVA, $F = 13.5$; $p < 0.001$). The mean total biomasses for five studied years were similar between deep sites: St 1 and St 2 (Table 3). Biomass in littoral was significantly

(t -test, $p < 0.001$) 4-fold higher than in deep sites. At the same time, the differences in mean biomasses between St 3 and St 4 were insignificant ($t_{3,4} = 0.15$; $p > 0.05$).

At all study sites we found significant differences in biomass and abundance between taxa (ANOVA, $p < 0.001$). *G. lacustris* with biomass of 0.8–5.5 $\text{g}\cdot\text{m}^{-2}$ accounted for 22–56 % of the total benthic biomass and bivalve mollusk *S. suecicum* (0.4–3.8 $\text{g}\cdot\text{m}^{-2}$) comprised 14–37 %. Also, *L. variegatus* contributed notably (0.1–0.9 $\text{g}\cdot\text{m}^{-2}$, 8–23 %). Biomass of littoral chironomid larvae was < 5 % of total in summer and increased to 15–32 % (up to 2.5 $\text{g}\cdot\text{m}^{-2}$) in the late August–October (Fig. 4). Aquatic insects (Trichoptera, Plecoptera, Megaloptera, and Ephemeroptera) were abundant in April–May (3.27 $\text{g}\cdot\text{m}^{-2}$, 33 %), and then decreased in their biomass abruptly (after May–June flight out of lake), varying 0.1–0.8 $\text{g}\cdot\text{m}^{-2}$. At deep sites 80 % of the total biomass was on account of the chironomid *S. coracina* (St 1) and the amphipod *M. affinis* (St 2). Seasonal differences in the biomass and abundance of taxa were insignificant at St 1 (ANOVA, $F = 0.93$ – 1.69 ; $p > 0.05$) and St 2 ($F = 1.31$ – 1.62 ; $p > 0.05$), while they were significant at St 3 and St 4 (biomass: $F = 2.41$ – 3.06 ; $p = 0.001$ – 0.002 ; abundance: $F = 2.89$ – 6.09 ; $p < 0.001$).

Excretion rate

Direct positive relationships were observed between the individual excretion rates (P_{exc}) and the individual weight of animals. For example, P_{exc} varied between 0.03 and 0.15 $\mu\text{g P h}^{-1}$ for *G. lacustris* with 45–128 mg of body wet weight (WW) and 0.1–40.35 $\mu\text{g P h}^{-1}$ for 150–420 mg *G. loricatus*. The correlation coefficient between P_{exc} and body weight of *S. coracina* was low (0.26) and insignificant. For other taxa P_{exc} –WW correlations were highly significant. P_{exc} –WW relationships were approximated by a power function (Fig. 5): 1) *S. suecicum* $P_{\text{exc}} = 0.03 \text{ WW}^{0.81}$, $n = 12$, $R^2 = 0.63$; 2) *L. stagnalis* $P_{\text{exc}} = 0.0003 \text{ WW}^{1.32}$, $n = 8$, $R^2 = 0.94$; 3) *G. lacustris* $P_{\text{exc}} = 0.196 \text{ WW}^{0.66}$, $n = 34$, $R^2 = 0.34$; 4) *G. loricatus* $P_{\text{exc}} = 0.073 \text{ WW}^{0.82}$, $n = 40$, $R^2 = 0.89$; 5) *M. affinis* $P_{\text{exc}} = 0.086 \text{ WW}^{0.74}$, $n = 18$, $R^2 = 0.81$; 6) *L. variegatus* $P_{\text{exc}} = 0.041 \text{ WW}^{0.88}$, $n = 16$, $R^2 = 0.73$; 7) *S. coracina* $P_{\text{exc}} = 0.05 \text{ WW}^{0.88}$, $n = 35$, $R^2 = 0.16$.

Regression lines for SRP excreted by *G. lacustris*, *G. loricatus*, and *L. variegatus* differ significantly according to F-test ($p < 0.001$). Also, F-test revealed significant differences ($p < 0.001$) in dispersions between

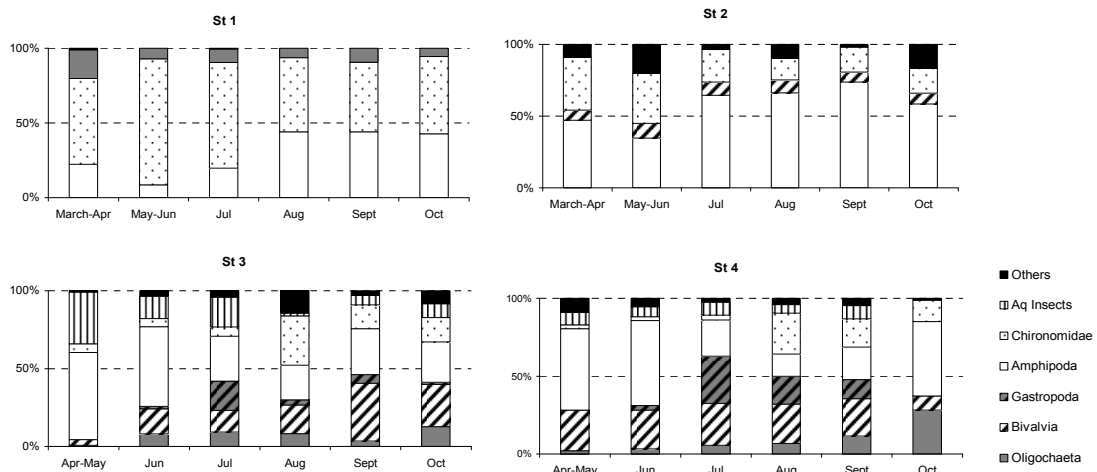


Fig. 4. Contribution (%) of various taxa to the total biomass of macrozoobenthos at four study sites of Lake Krivoe

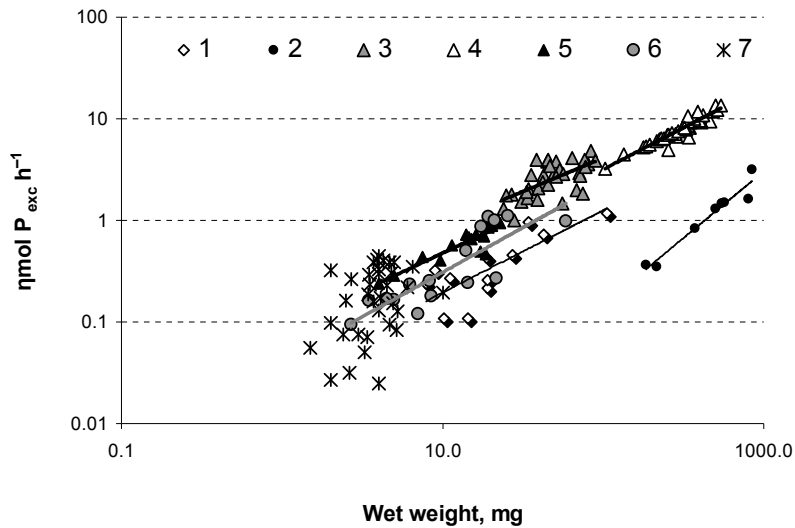


Fig. 5. Phosphorus excretion rate ($P\text{-PO}_4$; $\mu\text{mol P h}^{-1}$) by diverse benthic animals versa their individual wet weight (WW, mg) at water temperature of 10 °C: 1 – *Sphaerium suecicum*; 2 – *Lymnaea stagnalis*; 3 – *Gammarus lacustris*; 4 – *Gammaracanthus loricatus*; 5 – *Monoporeia affinis*; 6 – *Lumbriculus variegatus*; 7 – *Sergentia coracina*

L. stagnalis and *L. variegatus* as well as between all amphipods. There are no any significant differences in regression lines of two pairs of species: *L. stagnalis* and *S. suecicum* ($F = 0.71$, ns, $p > 0.05$), and *M. affinis* and *L. variegatus* ($F = 0.43$; ns, $p > 0.05$). There were significant differences in dispersions between variables in *S. suecicum* and *G. loricatus* ($F = 6.38$; $p < 0.001$). Also, slopes and intercepts of regression lines differed between *S. suecicum* versus *M. affinis*, *G. lacustris*, and *L. variegatus* ($t_{\text{slopes}} = 3.32\text{--}4.23$; $p < 0.05$), although their dispersions had no significant differences ($F = 0.04\text{--}1.55$; $p > 0.05$).

The average dry body weight (DW) and mass specific excretion rates (P_{exc}/DW) of animals differed between taxa (Table 4). Amphipods (*M. affinis*, *G. lacustris*) and chironomids tended to excrete SRP at significantly higher rates ($0.23\text{--}0.27 \mu\text{mol mg}^{-1} \text{h}^{-1}$ at 10 °C) than oligochaetes (0.17), and mollusks (0.01–0.04). The gastropod *L. stagnalis* tended to have the lowest SRP excretion rate as a result of highest body mass of the animals because of massive

shell. Water temperature was found as the main determinant factor for SRP excretion activity in animals. Coefficient Q_{10} in the temperature range of 10–20 °C varied slightly between species (Table 4).

The average values of P efflux caused by benthic excretion at study sites of the lake are presented on Fig. 6 (seasonal changes). The P efflux was significantly (F -test, $p < 0.01$) 5–6 times greater in littoral than in deep sites. It was lowest at St 1 ($0.3\text{--}1.62 \mu\text{mol m}^{-2} \text{d}^{-1}$) and highest at St 4 ($2.2\text{--}14.4 \mu\text{mol m}^{-2} \text{d}^{-1}$). Differences in average P efflux between littoral sites as well as between deep sites were insignificant ($p > 0.05$; Table 3). At the same time, littoral benthos contributed 57–62 % to the total P-efflux ($2.5 \mu\text{mol m}^{-2} \text{d}^{-1}$), in spite of the littoral area (0–3 m) consists only 20 % of the total lake area. The approximate values of the total P-flux on whole bottom area in Lake Krivoe ranged seasonally from 299 to 2332 averaging 1240 mmol d^{-1} .

The littoral amphipod *G. lacustris* and the profundal amphipod *M. affinis* were

Table 4. Min-Max and mean (\pm SE) body weight (DW, mg), mass-specific excretion rates of soluble reactive phosphorus (P_{exc}/DW , $\mu\text{mol mg}^{-1} \text{h}^{-1}$) at 10 °C, and the coefficient Q_{10} for SRP excretion rate of studied species from Lake Krivoe

Species	DW	P_{exc}/DW	Q_{10}
<i>Lumbriculus variegatus</i>	0.7–11.7	0.08–0.28	2.1
	3.0 \pm 0.7	0.17 \pm 0.02	
<i>Sphaerium suecicum</i>	1.8–169.0	0.02–0.17	2.0
	68.5 \pm 17.9	0.04 \pm 0.01	
<i>Lymnaea stagnalis</i>	36.0–169.0	0.01–0.02	3.0
	101.1 \pm 17.2	0.01 \pm 0.00	
<i>Monoporeia affinis</i>	0.8–4.6	0.10–0.30	2.6
	3.0 \pm 0.2	0.23 \pm 0.01	
<i>Gammarus lacustris</i>	4.7–15.3	0.12–0.44	2.6
	10.2 \pm 0.6	0.27 \pm 0.01	
<i>Gammaracanthus loricatus</i>	20.6–109.0	0.09–0.17	2.3
	64.0 \pm 3.3	0.13 \pm 0.01	
<i>Sergentia coracina</i>	0.3–2.0	0.03–0.81	2.3
	0.8 \pm 0.1	0.27 \pm 0.03	

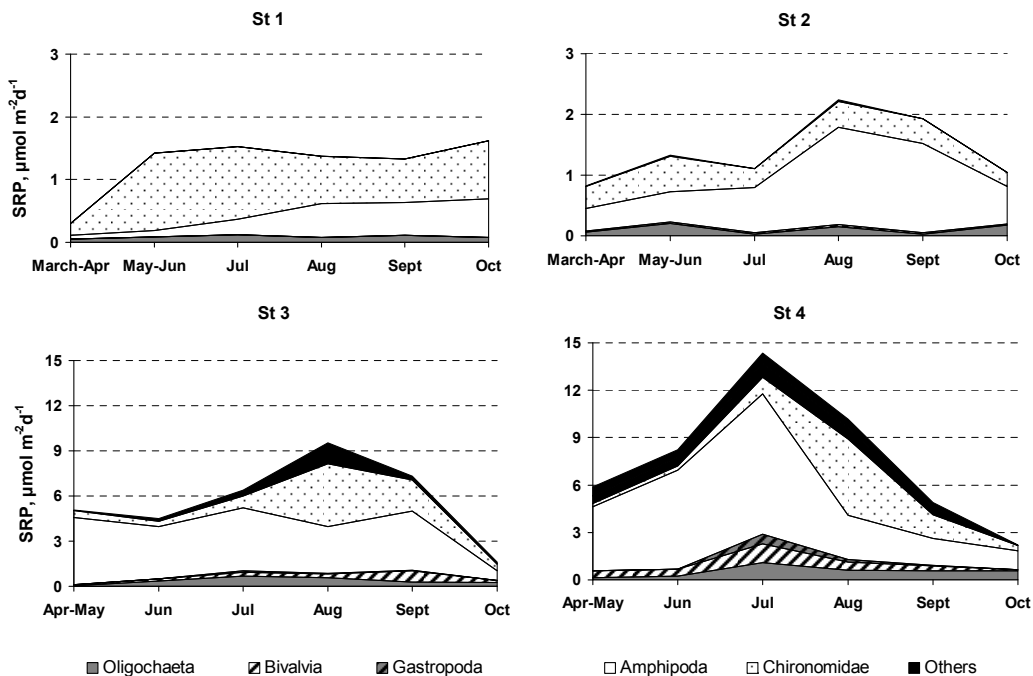


Fig. 6. SRP excretion fluxes ($\mu\text{mol m}^{-2} \text{d}^{-1}$) of various macrobenthic taxa at four study sites of Lake Krivoe during season (mean for 2009-2013). Contribution of megabenthic *Gammaracanthus loricatus* to the SRP excretion flux is not included

the main contributors (up to 80 %) to the P efflux among macrobenthic invertebrates at sites 2-4 (1.6–8.9 $\mu\text{mol m}^{-2} \text{d}^{-1}$). At the

deepest St 1 chironomids contributed up to 70 % (0.2–1.2 $\mu\text{mol m}^{-2} \text{d}^{-1}$) of the total flux but role of amphipods (*M. affinis*) declined

(0.1–0.6 $\mu\text{mol m}^{-2} \text{d}^{-1}$). At the same time, another megabenthic amphipod *G. loricatus* excreted 2.1–10.4 $\mu\text{mol m}^{-2} \text{d}^{-1}$ of SRP at St 1.

Activity of alkaline phosphatase

Relationships between the extracellular alkaline phosphatase activity (APA) and number and wet mass of amphipods in treatments are shown in Fig. 7. The activity of APA increased 3–8 times due to activity of amphipods (*G. lacustris*), comparing to the control. We found positive correlations between the activity of this enzyme and the abundance and the biomass of animals but correlation coefficient was high for APA versus abundance ($R = 0.83$; $n = 12$, $p < 0.05$) and insignificant in the case of APA versus biomass ($R = 0.54$; $n = 12$, $p > 0.05$). P excretion rate of the amphipod correlated positively with biomass of animals ($R = 0.78$; $n = 12$, $p < 0.05$) and with APA ($R = 0.86$; $n = 12$, $p < 0.05$).

Discussion

Positive relationships between P excretion rate and body mass of organisms are confirmed by this study as well as by other empirical data for different taxa: fish, zooplanktonic and benthic organisms (Johannes, 1964; Gutelmakher, 1981; Andersson et al., 1988; Schindler, Eby, 1997; Griffiths, 2006). We found differences in individual P excretion rates between taxa of high taxonomic ranks (class, order or family), such as Oligochaeta, Mollusca, and Chironomidae. Also, within order Amphipoda we found interspecies differences in P excretion rates between littoral *G. lacustris* and deepwater *G. loricatus*. Most likely, these differences related to differences in specific metabolic rates between these species. Conroy et al. (2005) also found differences in P excretion rates between species of the genus *Dreissena*.

Amphipods were main contributor of P efflux from the bottom to water in studied lake, where 70–90 % of P released due to excretion of

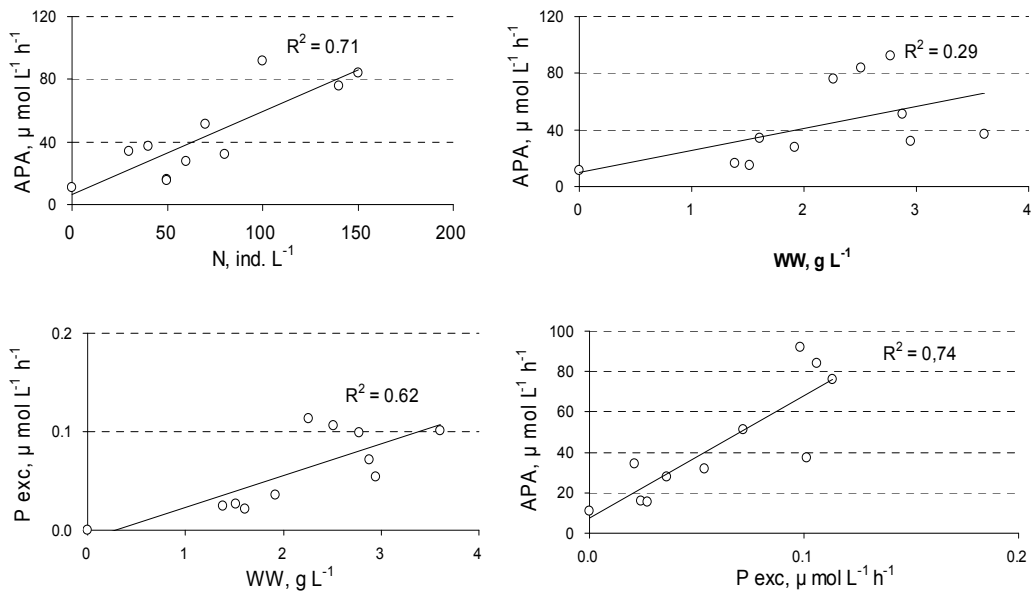


Fig. 7. Relationships between the extracellular alkaline phosphatase activity (APA) and abundance (N) and biomass (WW) of amphipods in treatments, and between soluble reactive phosphorus, excreted by amphipods (P_{exc}) and their biomass and APA. R^2 is coefficient of determination, $n = 12$

amphipods and rest on account of other taxa. In contrast, in eutrophic lakes (Acton, Donghu) P fluxes in oxic sites were determined, as a rule, by excretory activity of chironomids and tubificids (Devine, Vanni, 2002; Ji et al., 2011).

The variations in the P excretion rates are not only relate to characteristics of individual species/taxa but also reflect the character of environments. The obtained data confirmed that level of P excretion rate in benthic animals depends on water temperature. These relationships cause the seasonal differences in P fluxes that were found also in other studies of zooplankton (Oude, Gulati, 1988) and zoobenthos (Devine and Vanni, 2002; Postolache et al., 2006).

The highest rate of the P efflux by excretion of benthic invertebrates was recorded in the littoral of studied lake and the lowest in the central sites. The greater P fluxes at littoral sites in comparison with central sites were also found in other lakes (Devine, Vanni, 2002; Ji et al. 2011, 2015). Difference in P flux between central and littoral sites in small lakes is obviously the result of spatial differences in physical and chemical traits, trophicity, and zoobenthic biomass.

Mobile benthic animals such as amphipods occurred to be able to influence the activity of extracellular alkaline phosphatase, raising its level in the water. These enzymes are produced by bacteria and algae and can catalyze the hydrolysis of soluble organic phosphorus, probable excreted by aquatic animals. It is widely known that activities and ratios of commonly measured enzymes can be used as indicators of microbial nutrient demand (Crowther et al., 2011).

Influence of invertebrates on the extracellular enzymes, which are responsible for the decomposition of organic matter and the cycling of nutrients, was found as notable in some other cases. Burrowing mangrove crabs could directly impact the nutrient cycling in mangrove sediment, thereby accordingly changing the

microbial activity including enzymatic activity and microbial abundance (Luo, Gu, 2016). Extracellular enzyme activity is not only affected by environmental conditions but, also, reflects the available resources for algae and bacteria.

Animal excretory products could be not only in forms of DIP, but also dissolved organic P (DOP) and even particulate P fractions; and they may be highly bioavailable (Karl, Björkman, 2002; Stepanauskas et al., 2002; Nausch, Nausch, 2007). Plankton algae and bacteria producing their own phosphatases can facilitate liberation of bioavailable P from DOP compounds by phosphatase-catalysed hydrolysis (Wetzel, 2001; Chrost, Siuda, 2002). Phosphatase activity commonly occurred as high in environment, where ambient P is scarce (Healey, Hendzel, 1980; Pettersson, 1980; Gage, Gorham, 1985) as well as in the hyper-eutrophic ecosystem (urban Lake Donghu) at ample P concentrations (Zhou et al., 2007; Cao et al., 2005). Thus, the high activity of phosphatase in the water due to excretory activity of benthic organisms may testify about P acquisition (Sinsabaugh et al., 2012) and high bioavailability of excreted phosphates, facilitating of bacteria and algae growth and important for P cycle in water.

Conclusion

Despite the fact that phosphorus is the most studied of nutrients in the freshwater ecosystems, this study provides new evidences that benthic invertebrates can affect the internal phosphorus load and biomass of primary producers in the oligotrophic system. Mobile benthic invertebrates (amphipod crustaceans) were found as the most important contributor of phosphates excreted by benthic invertebrates due to their ability to move from bottom sediments to water and enrich the water column by this nutrient. The main flow of excreted phosphorus passes through the littoral zone of oligotrophic

lake, elevating notably in warm period. Direct relationships, recorded between phosphorus excreted by animals and activity of extracellular phosphatase, indicate about high bioavailability of excreted compounds and important role of consumers in P-cycling but mechanisms of these interactions need further investigations and understanding.

We thank staff of the White Sea Biological Station “Kartesh” (ZIN RAS) for providing facilities and help during field and experimental works and Dr. E.S. Kravchuk (Institute of Biophysics SB RAS, Krasnoyarsk, Russia) for useful comments on this paper. This work presents results of the Sino-Russian cooperation (grants from the National Natural Science Foundation of China 40911120038 and Russian Foundation for Basic Research 08-04-92217-GFEN_a) between Laboratory of Freshwater and Experimental hydrobiology (Russian Academy of Sciences, Zoological Institute, St. Petersburg, Russia) and Key Laboratory of Algal Biology (Chinese Academy of Sciences, Institute of Hydrobiology, Wuhan, China).

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