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Factors influencing phosphorus regeneration by lake zooplankton—An experimental approach

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ABSTRACT

Phosphorus and nitrogen concentrations in the trophogenic zone in lakes are main factors determining the trophic state of lakes. The goal of this work was to assess the impacts of enrichment of lake water with nutrients, as well as the presence of large crustaceans, *Dreissena polymorpha* and fish on the process of phosphorus regeneration by zooplankton (rotifers and crustaceans) in experimental mesocosms. The experiment was carried out for 32 days and consisted of 12 treatments, each replicated in triplicate. The mesocosms were filled with 270 L of natural pelagic water from the eutrophic Lake Mikołajskie and kept on the shore of the lake. Fish did not to have any impact on phosphorus regeneration rates in any of the treatments, whereas the presence of bivalves increased phosphorus regeneration rates. Phosphorus regeneration rates did not affect phosphorus sedimentation in any of the treatments, but the loss of total phosphorus and its increased regeneration rates resulted in markedly decreasing turnover times of phosphorus in all treatments of the experiment. Nutrient enrichment mainly affected phosphorus regeneration by phytophagous and predatory crustaceans and had no impact on phosphorus regeneration by rotifers when large Cladocera were absent. Processes of phosphorus regeneration and uptake included in internal phosphorus cycling were under strong influence of biological factors, which acted quickly, through the changing trophic structure of zooplankton communities, which in turn changed the flow of phosphorus in trophic chains.

1. Introduction

The rate of phosphorus (P) input to the trophogenic layer in lakes is the main factor that determines the rate of primary production (Andersson et al., 1988; Hudson et al., 1999; Pilcher et al., 2017) and thus, the trophic state of lakes (Ejsmont-Karabin, 1983). Two main cycles of phosphorus occur in lakes: a slower annual cycle that involves P sedimentation with organic and mineral particles and P transport to the trophogenic zone during spring and autumn circulation. P release from lake sediments is mostly controlled by oxygen concentrations, however some observations suggest that the process is much more complicated (Hupfer and Lewandowski, 2008). Two main processes are important for the P release from sediments: (1) anaerobic dissolution of iron bound P, and (2) mineralisation of settled organic particles Song and Burgin, 2017). The second cycle, taking place mainly in the trophogenic zone, has a much faster P flow rate, and includes P uptake and assimilation by producers, consumption of P built into the biomass of algae and bacteria by herbivores and bacterivores and remineralization of nutrients in a process of P excretion by zooplankton. Both of the cycles are interconnected as the pool of P and its sedimentation depends on the rate of its regeneration and the rate of P regeneration is influenced by inputs of nutrients into the inner cycle (Ejsmont-Karabin, 1983).

The relative importance of P as a limiting factor for primary production depends in part on zooplankton species composition and thus on nutrient storage and recycling (Carpenter et al., 1992). Nutrient recycling plays an essential role in the maintenance of the functioning of pelagic systems (Carrillo et al., 1995; Atkinson et al., 2017). In general, in lakes of low trophy, the input of mineral P from its regeneration by zooplankton and P utilization by phyto- and bacterioplankton are more or less balanced, whereas in highly eutrophic lakes regeneration usually exceeds uptake of phosphorus in summer (Ejsmont-Karabin et al., 1983). In the course of eutrophication, a structural change of zooplankton towards an increased abundance of

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bacterivores and decreased individual weights of rotifers and crustaceans (Ejsmont-Karabin, 2012; Ejsmont-Karabin and Karabin, 2013) leads to increased rate of P regeneration, whereas high abundance of large daphnids may lead to decrease in P regeneration rate and increase in P sedimentation rate (Rychla et al., 2014).

We hypothesize that P regeneration rates can respond to the effects of environmental stress factors at low and high trophic levels differently. The most common environmental factors that can affect zooplankton species structure and, consequently, P regeneration rates are variation in fish pressure and introduction of alien species in zooplankton communities and ecosytems. Large bodied zooplankton and *Dreissena polymorpha* are effective filrators and could affect zooplankton community structure bottom-up. Fish on the contrary is expected to regulate zooplankton community top-down. Nutrient enrichment can modify top-down and bottom up controls.

The goal of our study was to assess how alien large-bodied zooplankton, *Dreissena polymorpha* and fish influence, individually and in combinations, the P regeneration rates by different trophic groups of zooplankton at two levels of trophy.

2. Methods

2.1. Mesocosm design and sampling

The experiment was carried out from 31 July to 1 September 2012 and consisted of 12 treatments, each replicated in triplicate. Mesocosms (0.94 × 0.64 × 0.50 m; 300 L, food safe, high density polyethylene-HDPE, containers) were filled with 270 L of natural water taken from the pelagic zone, 1 m below surface of the eutrophic Lake Mikołajskie (Masurian Lake District, northeastern Poland; area 498 ha) and kept on the lake shore.

The set of treatments consisted of a control, i.e. unfiltered lake water that included small cladocerans *Chydorus sphaericus, Bosmina coregoni, Bosmina longirostris, Ceriodaphnia pulchella* and copepods *Eudiaptomus gracilis, Eudiaptomus graciloides, Mesocyclops leuckarti, Thermocyclops oithonoides* and a natural community of rotifers (C), and lake water with the addition of: alien species of crustaceans (AS), *Dreissena polymorpha* (DP), nutrients (N), fish (F), alien species plus *Dreissena* (ASDP), fish plus alien species (FAS), nutrients plus alien species (NAS), nutrients plus *Dreissena* (NDP), nutrients plus fish (NF), nutrients plus *Dreissena* and alien species (NDPAS), nutrients plus fish and alien species (NFAS).

The alien species treatment was a mixture of crustaceans added to each of 18 tanks: 500 ind. of *Daphnia pulex* Leydig (1.85 ind. L^{-1}) and 140 ind. of *Simocephalus vetulus* (O.F. Müller) (0.52 ind. L^{-1}). These species were not present in the source water (thus, they were alien to Lake Mikołajskie). They were collected from neighboring small waterbodies where they were found in densities similar to those added to the experiment (personal observation) and separated from the remaining plankton in the laboratory. They were added as inoculum with expectation that they would develop in mesocosms and become abundant.

Ruffe, *Gymnocephalus cernua,* were caught and placed in 5-L plastic boxes with large slots on 31 July so that water and plankton could pass through but the fish could not. The boxes were suspended inside 12 mesocosms. The fish were let out of the box for an hour to feed freely each day and returned to the box when they were fed. The boxes were used to limit predation on zooplankton. The total length (*longitude totalis*) of fish in one tank was 8.3 ± 0.4 cm. Fish of that size (ca. 1.0 cm) feed on copepods and cladocerans (Rezsu and Specziár, 2006). Watching fish during their “walks” confirmed that they were consuming large zooplankton.

Dreissena polymorpha, zebra mussels, were transported from Lake Boczne to the field station in coolers. They were added to the mesocosms within 24 h of collection. The mussels were introduced into 4 *Dreissena* treatments (12 mesocosms) in amount of 250 g per m^2 (i.e. about 200 ind. per tank or 0.75 ind. L^{-1} which is consistent with previous mesocosms experiments with *Dreissena*).

Water in 6 nutrient treatments (18 mesocosms) was enriched with 1.728 mg l^{-1} $N\text{-}NO_3$, 0.192 mg l^{-1} $N\text{-}NH_4$ and 0.120 mg l^{-1} $P\text{-}PO_4$. Nutrients were added at a ratio of 16:1 N:P to match the Redfield ratio. Phosphorus concentrations were determined spectrophotometrically with the molybdenum blue method (Golterman and Clymo, 1978).

After careful mixing the water in each mesocosm, 1-l samples were collected on days 1 (immediately after addition of AS, DP, N and F), 12, 22 and 32 of the experiment. The samples were fixed with Lugol’s solution, condensed on a 30 µm mesh-size plankton net, and again fixed in 2% formalin.

Individual body-weights were determined based on the relationships between body length and body weight for each crustacean (Balushkina and Vinberg, 1979) and rotifer species (Ejsmont-Karabin, 1998). Dry weight was estimated as suggested by Bottrell et al. (1976), i.e. the rate of dry to wet weight was 3.9% for Asplanchna and 10% for the remaining rotifers.

The rate of P excretion by animals was assessed using the regression equations obtained for animals feeding at the presence of food in concentrations typical of eutrophic lakes:

$$E = 0{,}0154\ e^{0{,}096T}\ DW^{-1{,}27}$$ for rotifers

$$E = 0{,}519\ e^{0{,}039T}\ DW^{-0{,}230}$$ for cladocerans

and

$$E = 0{,}299\ e^{0{,}039T}\ DW^{-0{,}645}$$ for copepods,

where: E = excretion rate in µg P mg $DW^{-1}\,h^{-1}$, T = temperature in °C; DW = dry weight in µg (Ejsmont-Karabin, 1984). The data used in the equations were abundance and biomass of all rotifer and crustacean species and their developmental stages obtained from samples collected during the experiment from appropriate mesocosms. The excretions rates were calculated for the particular species and total excretion rates were found as sum of the values.

The rate of P net sedimentation was estimated indirectly from the difference in total P concentrations on the last and first day of the experiment.

To assess the pools of phosphorus consumed and regenerated by zooplankton, P regeneration rates were calculated separately for trophic groups (guilds), including detrivores (small rotifers of the genera *Anuraeopsis, Keratella* and *Lecane*), herbivores (copepods, except adult ones, calanoids and rotifers of the genera *Polyarthra, Synchaeta* and *Trichocerca*) according to Pourriot (1977), omnivores (filter-feeders, mostly Cladocera with both bacteria and small algae in their diet, (DeMott, 1982; Kerfoot and Kirk, 1991) and predators (adult cyclopoids).

2.2. Statistical analyses

We used multivariate analysis of variance for repeated measures (RM-MANOVA) and two-factor repeated-measures analysis of variance with equal and unequal numbers of replicates in the cells (RM-ANOVA) to determine how the experimental treatments affected biological parameters. Multivariate analysis assessed the impact of the treatments on dependent variables: ROTherb (herbivorous rotifers), ROTdet (detritovorous rotifers); ROTomn (omnivorous rotifers); CRUherb (herbivorous crustaceans), CRUomn (omnivorous crustaceans), CRUpred (predatory crustaceans); Ptot (total phosphorus), P-PO4 (phosphates); N-NH4 (ammonium), N-NO3 (nitrates). If MANOVA based on criteria Wilks's lambda, Pillai's trace, Hotelling- Lawley trace and Roy's largest root rejected the null hypothesis, we used one-dimensional two-factor analysis of variance for each dependent variable to determine which average values of variables significantly differed from each other. If the results of MANOVA after using all the above criteria varied, we gave the preference to Pillai's trace since this test is the most robust (Olson, 1974, 1976, 1979; Zar, 2010). If the sample sizes were not equal, we