

## Effects of the zebra mussel, an exotic freshwater species, on seston stoichiometry

Rahmat Naddafi and Kurt Pettersson

Department of Ecology and Evolution/Erken Laboratory, Evolutionary Biology Centre, Uppsala University, Norr Malma 4200, 76173 Norrtälje, Sweden

Peter Eklöv

Department of Ecology and Evolution/Limnology, Evolutionary Biology Centre, Uppsala University, Norbyvägen 20, SE-752 36 Uppsala, Sweden

### Abstract

We examined the effect of the zebra mussel, *Dreissena polymorpha*, an exotic species, on seston stoichiometry by conducting laboratory experiments in which we varied nutrient composition of seston and mussels over time. Zebra mussels altered the stoichiometry of seston through removal of particulate organic nutrients and changed the stoichiometry of the dissolved nutrient pool through nutrient excretion. Grazers had stronger effects on carbon:phosphorus (C:P) and nitrogen (N):P ratios than on the C:N ratio of seston. Elemental residence time in tissue and high mass-specific nutrient excretion by small mussels caused small mussels to be more efficient nutrient recyclers than larger mussels. Zebra mussels reduced P availability through enhancing C:P and N:P molar ratios of seston during the period extending from June to August, when P was limited in the lake, and increased the C:N molar ratio of seston in June, when N was at the minimum level in the lake. C:P and N:P molar ratios for zebra mussel tissue were higher in August and somewhat in September than in all other months. N was retained more efficiently than P in *Dreissena* tissue. Nutrient mass-specific uptake rate was higher than excretion rate by zebra mussels.

Ecologists are increasingly interested in understanding the balance of multiple chemical elements in ecological interactions using the ecological stoichiometry approach (Elser et al. 1996, 2000a; Hessen 1997). This is because stoichiometric imbalances between resources and consumers have been found to have implications both for consumer growth and for nutrient recycling in food webs and may constrain or change key ecosystem processes (Sturner and Hessen 1994; Elser and Urabe 1999; Sturner and Elser 2002). Measuring ecological stoichiometry has emerged as a powerful tool for understanding mechanisms and processes important for the structure, biodiversity, and function of aquatic communities. It has been applied to address important questions in ecology, including those related to population dynamics (Andersen et al. 2004), trophic interactions (Elser et al. 2000b), nutrient cycling, and food web dynamics (Elser and Hassett 1994; Elser and Urabe 1999; Sturner and Elser 2002).

Aquatic exotic species pose a serious threat to the economy, ecology, and biodiversity of aquatic ecosystems, in which their effects are difficult to invert or mitigate. The zebra mussel, *Dreissena polymorpha*, is an exotic species capable of altering community structure and ecosystem function of lakes through a large grazing capacity and

selective feeding (MacIsaac 1996; Naddafi et al. 2007b). In addition, *Dreissena* may have large ecosystem-level outcomes as a result of its ability to cycle phosphorus and/or nitrogen (Mellina et al. 1995; Arnott and Vanni 1996; Conroy et al. 2005).

Zebra mussels filter a large but variable size range (0.7  $\mu\text{m}$ –1.2 mm) of particles (Naddafi et al. 2007b) from the water column. Phosphorus (P), nitrogen (N), and carbon (C) associated with seston are entered into mussels' inhalant siphon and ingested. The ingested nutrients are either assimilated or egested. Egested nutrients deposited as feces may become available to primary producers through mixing or microbial decomposition. Assimilated nutrients are either sequestered at relatively constant concentrations in mussels' tissues and used for growth and reproduction (mussels as a nutrient sink) or excreted in dissolved inorganic form (mussels as a nutrient source), which can provide a substantial proportion of the nutrient demands of primary producers. In addition, the phytoplankton that are rejected in pseudofeces (Naddafi et al. 2007b) have higher access to nutrients because of lower remaining phytoplankton biomass. Consequently, zebra mussels may change resource supply to the prey through nutrient excretion and may enhance resource availability.

N and P can limit phytoplankton growth and the abundance of other primary producers. Further, the N and P ratio is an important aspect of resource-related indirect effects of consumers, and, thus, the ratio at which zebra mussels excrete N and P will determine the relative degree of N and P limitation, which in turn could affect algal composition (Smith 1983; Elser and Hassett 1994; Arnott and Vanni 1996). Evidence indicates that zebra mussels may shift the nutrient regimes in lakes. For example, both ammonia and nitrate have increased in the

### Acknowledgments

We thank Helena Enderskog, Olga Matzen, Jan Johansson, and Björn Mattsson for assistance in laboratory and fieldwork. We also greatly appreciate the comments of two anonymous reviewers, which improved this manuscript considerably.

Funding for this research was provided by the Malméns and the Olsson–Borgh foundations as well as Erken laboratory (R.N.) and The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (P.E.).

western basin of Lake Erie since it experienced a zebra mussel invasion (Holland et al. 1995; Makarewicz et al. 2000). In particular, ammonium is the preferred form of N for phytoplankton uptake, which can facilitate the growth of harmful algae such as cyanobacteria (Ward and Wetzel 1980). It has also been indicated that zebra mussels may decrease P limitation and increase N limitation of the phytoplankton by N:P excretion at levels below the Redfield ratio of 16:1 (Arnott and Vanni 1996). Such shift toward N limitation may result in dominance of cyanobacteria over other phytoplankton groups (Smith 1983).

The nutrient composition of food (phytoplankton) has high variability, whereas consumers' body mass stoichiometry is less variable (Elser and Urabe 1999; Sterner and Elser 2002; Liess and Hillebrand 2005). In addition, the mismatch between the nutrient composition of primary producers and the nutrient demand of consumers has prominent implications for nutrient recycling by consumers (Elser and Urabe 1999; Vanni 2002; Hillebrand et al. 2004). Nutrient excretion rate by an animal is positively correlated to the nutrient concentration in the food and negatively correlated to the nutrient content of body tissues (Vanni 2002). Because the concentrations of soluble and particulate nutrients differ over time in lakes, it is possible that zebra mussels will change the N:P ratio of soluble nutrients through homeostatic nutrient excretion with time. On the other hand, zebra mussels have the ability to decrease particulate organic phosphorus, particulate organic nitrogen, and particulate organic carbon (POC) (Johengen et al. 1995; James et al. 1997), thereby changing C:P, N:P, and C:N ratios of seston. Still, it is unclear how *Dreissena* affect seston stoichiometry by nutrient consumption.

We hypothesized that zebra mussels change seston C:N:P stoichiometry through nutrient consumption and alter stoichiometry of dissolved nutrient pools of lakes through nutrient excretion. First we examined whether the flux in nutrient caused by differences in zebra mussel consumption would lead to a variation in phytoplankton nutrient limitation. We then determined the importance of nutrient excretion as a potential mechanism controlling nutrient supply, phytoplankton community structure, and changes in the seston stoichiometry. In this context, tissue mass stoichiometry of mussels as well as N and P turnover time, relative to tissue content, were quantified.

Further, body size is an important factor in determining nutrient recycling rates and ratios by organisms (Peters 1983; Vanni et al. 2002). For example, Vanni et al. (2002) found that much of the interspecific variation in N:P excretion ratio among 26 fish and two amphibian species in a tropical stream in Venezuela was not only explained by body nutrient ratios (i.e., ecological stoichiometry) but also by body size. In addition, there is a negative relationship between zebra mussel growth rate and body size (R. Naddafi, K. Pettersson, and P. Eklöv unpubl.). On the other hand, fast-growing organisms have a large pool of P in ribosomal RNA, needed to meet the demand for protein synthesis to sustain high growth rates (Elser et al. 1996; Sterner and Elser 2002). Thus, small mussels would presumably have higher P demands and, thus, a higher N:P ratio in excretion. Consequently, different size

distributions of zebra mussels are supposed to have different effects on the function of the ecosystem, and we quantified effects of *Dreissena* on seston stoichiometry using three size-classes of mussels. To evaluate the effect of zebra mussels on seston stoichiometry, we conducted monthly laboratory experiments in which we measured nutrient composition of seston and mussels.

## Methods

The study was conducted in Lake Erken, a moderately deep meso-eutrophic (total P [Tot-P] 26.1–51.4  $\mu\text{g L}^{-1}$ ) and dimictic lake situated in southeast Sweden (59°51'N, 18°35'E). The lake has a surface area of approximately 24 km<sup>2</sup>, a mean depth of 9 m, and a maximum depth of 21 m. Zebra mussels invaded Lake Erken in mid-1975 and have spread rapidly since then (Naddafi et al. 2007a,b).

To measure particulate and soluble nutrient concentrations in Lake Erken, a weekly water sample was taken 700 m offshore the laboratory at the deepest point in the lake from April to September 2005. In addition, water sampling was performed once in January and December and twice in February, March, October, and November 2005. The samples were taken at 1, 5, 10, 15, and 19 m in depth. Water samples were combined for analysis of the concentrations of dissolved inorganic phosphorus ( $\text{PO}_4^{3-}\text{-P}$ ), dissolved inorganic nitrogen (DIN) ( $\text{NH}_4^+$  and  $\text{NO}_3^- + \text{NO}_2^-$ ), particulate phosphorus (PP), and particulate nitrogen (PN) (see below).

We performed our experiment in twelve 10-liter vessels at Erken laboratory (Norrtälje, Sweden) once a month during the period ranging from June to November 2005. We collected mussels for the experiment by scuba diving at one site in the eastern basin of Lake Erken (3-m depth). The mussels were placed in buckets filled with lake water and were transported to the laboratory. In the laboratory, we detached the mussels from their natural substrate by severing their byssal threads with a scalpel and brushed them clean to remove silt and algae adhering to the shells. The mussels were separated into the three size-classes: small (<14-mm shell length), medium (14–20-mm shell length), and large (>20-mm shell length) mussels.

To acclimate the zebra mussels to laboratory conditions, 300 healthy mussels (100 per size-class) were kept in three 60-liter aerated containers (filled with natural lake water) placed in a climate-controlled room at ambient lake temperature for almost 1 d prior to running the experiment each month (Arnott and Vanni 1996; Naddafi et al. 2007b). After the acclimation period, all mussels chosen for the experiment were visibly healthy and active (as indicated by comparing their filtration activity to that of recently collected mussels). The water in the containers was exchanged with fresh lake water after 24 h and also 2 h before the experiment began.

On day 1 of the experiment, 96 liters of lake water was filtered through a 100- $\mu\text{m}$  mesh net to remove most of the zooplankton and was then transferred to a separate container. After homogenization the water was poured into twelve 10-liter vessels (8 liters per vessel). Initial measurements confirmed that microzooplankton abun-

dance, phytoplankton biomass, and nutrient concentrations did not differ among the 12 vessels at the beginning of each experiment. The nine vessels were randomly assigned to three treatments each containing 20 individuals of small, medium, and large size-classes of zebra mussels, and each treatment was replicated three times. In addition, one treatment with three vessels containing lake water and no mussels served as a control to correct for changes in phytoplankton biomass due to zooplankton grazing and/or pigment degradation (Fanslow et al. 1995) and for any extraneous nutrient additions.

All vessels were placed in a climate room at the in situ temperature, and grazing or nutrient excretion experiments were run concurrently. A gentle aeration was done to keep the particles suspended in the water. Keeping the aerator filtration media near the water surface, gentle aeration as well as direct observation ensured that there was no/little resuspension of feces or pseudofeces deposited by the mussels during the experiments. Because our purpose was to investigate the effects of *Dreissena* on seston stoichiometry through conversion of particulate nutrients into soluble forms, we did not measure the nutrient content of fecal pellets generated during the experiment and excluded them from our measurements.

Immediately before the experiment started the water was sampled in the vessels to measure nutrient concentrations. The water sample was divided into five different subsamples for each replicate. First, an aliquot was used for the analysis of total phosphorus (TP) and total nitrogen (TN); second, an aliquot was used to measure total chlorophyll *a* (Chl *a*); third, an aliquot was used for the analysis of ammonium-nitrogen ( $\text{NH}_4^+$ -N), nitrate plus nitrite nitrogen ( $\text{NO}_3^-$   $\text{NO}_2^-$ -N), and phosphate-phosphorus ( $\text{PO}_4^{3-}$ -P); fourth, an aliquot was filtered on precombusted GF/C filters for the analysis of particulate C and particulate N; and fifth, an aliquot was filtered on precombusted GF/C filters for the analysis of particulate phosphorus.

The mussels were placed in the vessels and the experiment started when the inhalant and exhalant siphons were fully extended, which was generally within 3–5 min after the introduction of the mussels. Subsequent water samples were taken at 2 and 6 h after the mussels were added to the vessels. The first water sample (after 2 h) was immediately analyzed by delayed fluorescence excitation spectroscopy to determine total phytoplankton biomass, measured by Chl *a* (Naddafi et al. 2007a,b). The second water sample (after 6 h) was divided into subsamples in the same manner as prior to the experiment (*see above*) to measure all nutrient concentrations. A 2-h grazing period was chosen to avoid the the risk of chlorophyll concentration being below the detection limit at a longer experimental time period (Naddafi et al. 2007b). The 6-h excretion period was selected to allow enough nutrient excretion for measurable and replicable results (Gardner et al. 1995; Conroy et al. 2005) and low variability in excretion rate (Arnott and Vanni 1996). In addition, molar ratios of C:P, N:P, and C:N for seston stoichiometry were calculated initially and at the end of experiment (after 6 h).

Particulate C and particulate N of seston were measured simultaneously with a carbon-hydrogen-nitrogen (CHN)

analyzer (LECO CHN-932, Carlo-Erba Strumentazione), and particulate P of seston was measured as phosphate after hot hydrolysis with potassium persulfate (Grasshoff et al. 1983). The measured particle nutrients in our experiments can be considered as particulate organic nutrients, as demonstrated by previous experiments. In addition, we did not sample lake water on windy days, thereby avoiding the possibility that inorganic particles of sediment might be included in our seston samples. However, Lake Erken sediment contained no inorganic C and N particles and few inorganic P particles. Total N and dissolved nitrate plus nitrite were analyzed with the sulfanilamide method, dissolved ammonium with the Tecators method, and total P and dissolved phosphate with the ammonium-molybdate method (Grasshoff et al. 1983), in an auto-analyzer (FIA Flow Injection Analyzer).

At the end of the experiments, the mussels were measured (shell length, mm) with a vernier caliper (0.05 mm) to calculate dry tissue weight (mg) using a length-weight regression from Conroy et al. (2005) ( $W[\text{mg}] = 0.01213 L[\text{mm}]^{2.537}$ ) and to determine weight-specific clearance rates, changing rate of C:N:P stoichiometry of seston, and excretion rates.

To determine tissue nutrient content, 15 of each size-class (small, <14-mm shell length; medium, 14–20-mm shell length; large, >20-mm shell length) were collected monthly from the eastern basin of the lake. These mussels were cleaned using the same methods as for the experiments and were kept overnight in water without food to allow them to empty their guts. Individuals of each size-class were then placed separately in plastic tissue-culture plates and stored in the freezer for C, N, and P analysis (Liess and Hillebrand 2005). Specimens were freeze-dried to constant weight, and the dry mass of each individual was determined (Liess and Hillebrand 2005). After weighing, individual mussel shell length was measured and soft tissue was scraped off of the shells using a scalpel. Sample masses of 14 to 5,510  $\mu\text{g}$  were used for the analysis, depending on mussel size. Similar specific tissue parts were sampled for all specimens and were analyzed for C, N, and P tissue content using the same methods as for seston nutrient analyses. The same individual was used for the P analysis as for the CHN analysis. C:N, C:P, and N:P ratios were calculated in molar units.

*Data analysis*—Clearance rate, an indicator of mussel grazing activity, was calculated as the volume of water from which a mussel had removed all of the suspended particles per unit time. Specific clearance rates of zebra mussels were calculated separately for each size-class treatment and for each particle type based on the depletion of total Chl *a* (total phytoplankton biomass) in an 8-liter suspension for a 2-h period and based on the reduction of particulate P, particulate N, and particulate C in an 8-liter suspension for a 6-h period, according to the method of Coughlan (1969):

$$CR = \frac{V}{NT} \left\{ \ln \frac{C_0}{C_t} - \ln \frac{C'_0}{C'_t} \right\}$$

where  $V$  = the volume of lake water in the vessels

(8,000 mL);  $N$  = the weight of the 20 mussels (mg dry weight [dry weight (dry wt)]);  $T$  = the duration of the experiment (in hours);  $C_0$  = initial concentration ( $\mu\text{g L}^{-1}$ ) of each particle in the vessels with mussels;  $C_t$  = final concentration ( $\mu\text{g L}^{-1}$ ) of each particle in the vessels with mussels;  $C_0'$  = mean initial concentration of each particle in all control vessels of each size-class treatment; and  $C_t'$  = mean final concentration of each particle in all control vessels of each size-class treatment. The calculated clearance rate for all particles refers to gross clearance rate, because the feces and pseudofeces were not returned to the water column in our experiment (Bastviken et al. 1998).

Mass uptake rate of nutrients (mg of N, P, or C mg dry weight $^{-1}$  day $^{-1}$ ) by zebra mussels in Lake Erken was calculated as the clearance rate (mL mg dry wt $^{-1}$  h $^{-1}$ ) for each particle multiplied by the amount of that particle in volume unit of lake water ( $\mu\text{g mL}^{-1}$ ) multiplied by 24 h. We then calculated the ratio of the rates of the nutrient release to nutrient uptake among months and mussel size-classes.

To determine rates of soluble nutrient excretion, we divided the difference in the amount of nutrients in a vessel over the time interval, corrected for control rates, by the time interval. Multiplying the measured nutrient concentration by the vessel volume, dividing by the soft tissue biomass of 20 mussels, and multiplying by 24 h gave nutrient excretion rates as micrograms of N or P per milligram of dry weight per day (Conroy et al. 2005).

The calculated excretion rates should be considered to be “net” rather than “actual” rates because we did not measure nutrient absorption and excretion by phytoplankton, microzooplankton, and microbial (bacteria and fungi) communities. However, we homogenized the water well before transferring it to all vessels and assumed that all organisms' biomasses were equal in all aquaria. We observed that initial phytoplankton biomasses were identical in all vessels, which indicates that our homogenization was successful. Therefore, the starting condition was similar for all aquaria and our observed results assumed to be related to zebra mussel grazing and excretion.

We calculated the changing rate in the experiment of C:N:P molar ratios of seston. Changing rate was considered as the change in molar ratios of C:N, N:P, and C:P of seston in a vessel over time, corrected for control rates, divided by the total time interval. Then we divided the calculated changing rate by the soft tissue biomass of 20 mussels and multiplied by 24 h to express changing rate in molar ratios of C:P, N:P, and C:N for seston as unit per milligram dry weight per day. P and N turnover was determined as the time required to excrete nutrients in relation to content in tissue (Hatcher 1994) and was calculated as tissue content divided by excretion per day.

We analyzed month, size, and food type (Chl *a*, particulate P, particulate N, and particulate C) as main effects on mussel clearance rate using a three-way analysis of variance (ANOVA). We performed two-way ANOVAs with month and shell length as fixed factors, separately, for comparison of values of  $\text{PO}_4^{3-}\text{-P}$ ,  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{NO}_2^-\text{-N}$ , TP and TN concentrations, dissolved inorganic N:P molar ratio, and molar ratios of C:P, N:P, and C:N for seston

stoichiometry among treatment vessels that had been measured at the end of each experiment (after 6 h). We also employed two-way ANOVAs with size-class and month as variables to test the effect of month and size on nutrient excretion rate and C:N:P changing rate of seston by zebra mussels as well as on C:N:P stoichiometry of mussel tissues. We performed a one-way ANOVA to compare between particulate nutrient uptake rate and soluble nutrient excretion rate among size-classes for each month. Two-way ANOVAs were used to test the effect of month and size on the ratio of the rates of nutrient release to nutrient uptake. All analyses were followed by a Tukey test for multiple comparisons. A *t*-test was used to test whether the excretion rate of soluble nutrients or uptake rate of particle nutrients or changing rate of seston were significantly different from zero, separately for each month. All data were tested for homogeneity of variances and were  $\log_{10}$ -transformed if necessary. All statistical tests were performed with software package SPSS 14.0. Statistical significance was accepted at the  $p < 0.05$  level.

## Results

*Temperature, nutrient concentration, and seston stoichiometry*—Temperature and concentrations of TP, TN, PP, PN, particulate carbon (PC),  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{NO}_2^-\text{-N}$ ,  $\text{PO}_4^{3-}\text{-P}$ , Chl *a*, and molar ratios of C:N, C:P, and C:N for seston stoichiometry varied among experiments (Table 1). The concentration of particulate nutrients, including PP, PN, and PC, was high during June through August, whereas the concentration of soluble nutrients such as  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{NO}_2^-\text{-N}$ , and  $\text{PO}_4^{3-}\text{-P}$  was high during September through November (Table 1). Variation in TP and TN concentrations was due to different concentrations of particulate and soluble nutrients (P and N) (Table 1). In addition, seston C:P, N:P, and C:N molar ratios varied over time as a result of different particulate organic nutrient concentrations (Table 1).

At the end of experiment, the concentrations of PP, PN, PC, TP, TN, Chl *a*,  $\text{PO}_4^{3-}\text{-P}$ ,  $\text{NH}_4^+\text{-N}$ , and  $\text{NO}_3^-\text{NO}_2^-\text{-N}$  in control treatments (6 h, but 2 h for Chl *a*) did not differ from those at the beginning of the experiment (initial concentrations) (one-way ANOVA, all  $F_{1,34} < 1.8$  and all  $p > 0.2$ ), indicating that sedimentation as well as nutrient excretion and uptake by other organisms during our experiments was not important.

In Lake Erken,  $\text{PO}_4^{3-}\text{-P}$  concentration was lower during April through August than during January through March and September through November (one-way ANOVA,  $F_{2,47} = 27.3$ ,  $p < 0.001$ ). DIN concentration in both forms of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{NO}_2^-\text{-N}$  was relatively low during May through August, with the lowest value in June, and was relatively high during September through November (Table 1), but DIN was high in the form of  $\text{NO}_3^-\text{NO}_2^-\text{-N}$  during January through April. The high PP and PN concentrations observed in April and July through September were probably a result of diatoms and cyanobacteria blooms. The period of our experiment (June–November) involved both N and P limitation and availability in the lake (Table 1). Initial chlorophyll

Table 1. Temperature ( $^{\circ}\text{C}$ ), initial nutrient concentration, and seston stoichiometry in experimental vessels during June through November 2005. Concentrations of particulate phosphorus (PP), particulate nitrogen (PN), particulate carbon (PC), dissolved inorganic nitrogen (DIN), and phosphate in Lake Erken are also given.

Parameters ( $\mu\text{g L}^{-1}$ )	Months					
	Jun	Jul	Aug	Sep	Oct	Nov
T ( $^{\circ}\text{C}$ )	17.8	19.1	18.4	14	9.8	4
TP	16	27	15	22	35	27
TN	633	921	630	648	685	745
PP	5.1	8.4	5.6	4.6	4.5	2.4
PN	53.5	64.9	54.6	54.8	33.7	30.2
PC	392.7	393.7	356.0	321.8	247.3	212.1
$\text{NH}_4^+$ -N	14	9.0	9.0	17	34	5.0
$\text{NO}_3^-$ $\text{NO}_2^-$ -N	8.0	4.0	2.5	10.5	33.5	95
$\text{PO}_4^{3-}$ -P	3.0	3.0	1.0	15	25	18
Chl <i>a</i>	1.7	3.6	2.3	1.2	0.7	0.4
seston C:P	196.3	120.7	161.8	181.3	143.0	252.8
seston N:P	22.9	16.9	21.2	26.0	16.6	30.8
seston C:N	8.6	7.2	7.6	7.1	8.6	8.2
PP*	6.8	8.6	11.3	9.9	9.4	5.4
PN*	47.4	45.5	79.2	62.1	54.6	35.6
DIN*	9.9	25.9	50.8	77.6	72	104
$\text{PO}_4^{3-}$ -P*	2.7	4.9	10.2	19	26.5	23.5

T, temperature; TP, total phosphorus; TN, total nitrogen; Chl *a*, chlorophyll *a*.

concentrations in experimental vessels were highly correlated with PP ( $r^2 = 0.88$ ,  $n = 54$ ,  $p < 0.001$ ), PN ( $r^2 = 0.77$ ,  $n = 54$ ,  $p < 0.001$ ), and PC ( $r^2 = 0.69$ ,  $n = 54$ ,  $p < 0.001$ ).

**Clearance rate**—Clearance rates of zebra mussels varied among months, size-classes, and particle types (Table 2; Fig. 1). The mussels consistently cleared Chl *a* with the highest efficiency, and the clearance rate was higher for PP than for PN and PC (Table 2; Fig. 1). The regressions of clearance rate on temperature were significant for Chl *a* ( $r^2 = 0.28$ ,  $n = 54$ ,  $p < 0.001$ ) and PP ( $r^2 = 0.23$ ,  $n = 54$ ,  $p < 0.001$ ) but not for PN ( $r^2 = 0.007$ ,  $n = 54$ ,  $p > 0.05$ ) and PC ( $r^2 = 0.0005$ ,  $n = 54$ ,  $p > 0.05$ ).

The mussels exhibited high clearance rates during June through August when temperature varied between 17.8 $^{\circ}\text{C}$

and 19.1 $^{\circ}\text{C}$  (Tables 1, 2). However, a strong month  $\times$  particle interaction revealed that mussels cleared different particles among months (Table 2; Fig. 1). In general, small mussels cleared more particles per milligram dry weight than did medium and large mussels (Table 2; Fig. 1). The effect of size on mussel clearance rate differed across the month but not among particle types, as indicated by the strong size  $\times$  month interaction and the weak size  $\times$  food type interaction, respectively (Table 2). The nonsignificant month  $\times$  size  $\times$  food type interaction revealed that the effect of size on mussel clearance rate for all particle types was not time dependent (Table 2).

**Changes in seston stoichiometry**—Nutrient concentrations and dissolved inorganic N:P ratio at the end of experiment (6 h) varied significantly among months ( $p < 0.001$ ; Table 3), possibly as a result of monthly differences between their initial amounts (Table 1). The significant month  $\times$  treatment interactions revealed that the changes in nutrient concentrations ( $\text{NO}_3^-$   $\text{NO}_2^-$ -N,  $\text{PO}_4^{3-}$ -P,  $\text{NH}_4^+$ -N) and molar ratios of dissolved N:P, particulate N:P, C:P, and C:N were affected differently across the month among treatments ( $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$ ; Tables 3, 4). There was no significant difference between start and end values of TP and TN ( $p > 0.05$ ; Table 3).

**Changes in seston stoichiometry through nutrient consumption**—Molar ratios of C:P, N:P, and C:N in seston at the end of the experiment varied monthly (Table 4; Fig. 2), probably as a result of various values at the beginning of the experiment (Table 1) and different zebra mussel clearance rate of PP, PN, and PC over the months (Fig. 1B–D). These ratios differed among treatment (Table 4), indicating that *Dreissena* changed the molar ratios

Table 2. Three-way ANOVA for the effect of month, size, and food type (chlorophyll *a* [Chl *a*], particulate phosphorus [PP], particulate nitrogen [PN], particulate carbon [PC]) on zebra mussel clearance rates. For each row, similar superscripted lowercase letters (a, b, and c) indicate groups that are not significantly different ( $p > 0.05$ , Tukey test).

Source of variation	df	F	Tukey test results
Month	5	19.7 (<0.001)	Jun <sup>a</sup> ; Jul <sup>a</sup> ; Aug <sup>a</sup> ; Sep <sup>b</sup> ; Oct <sup>b</sup> ; Nov <sup>b</sup>
Size	2	95.8 (<0.001)	Small <sup>a</sup> ; medium <sup>b</sup> ; large <sup>c</sup>
Food type	3	262.6 (<0.001)	Chl <i>a</i> <sup>a</sup> ; PP <sup>b</sup> ; PN <sup>c</sup> ; PC <sup>c</sup>
Month $\times$ size	10	3.7 (<0.001)	
Month $\times$ food type	15	12.8 (<0.001)	
Size $\times$ food type	6	0.7 (0.6)	
Month $\times$ size $\times$ food type	30	1.2 (0.2)	
Error	144		

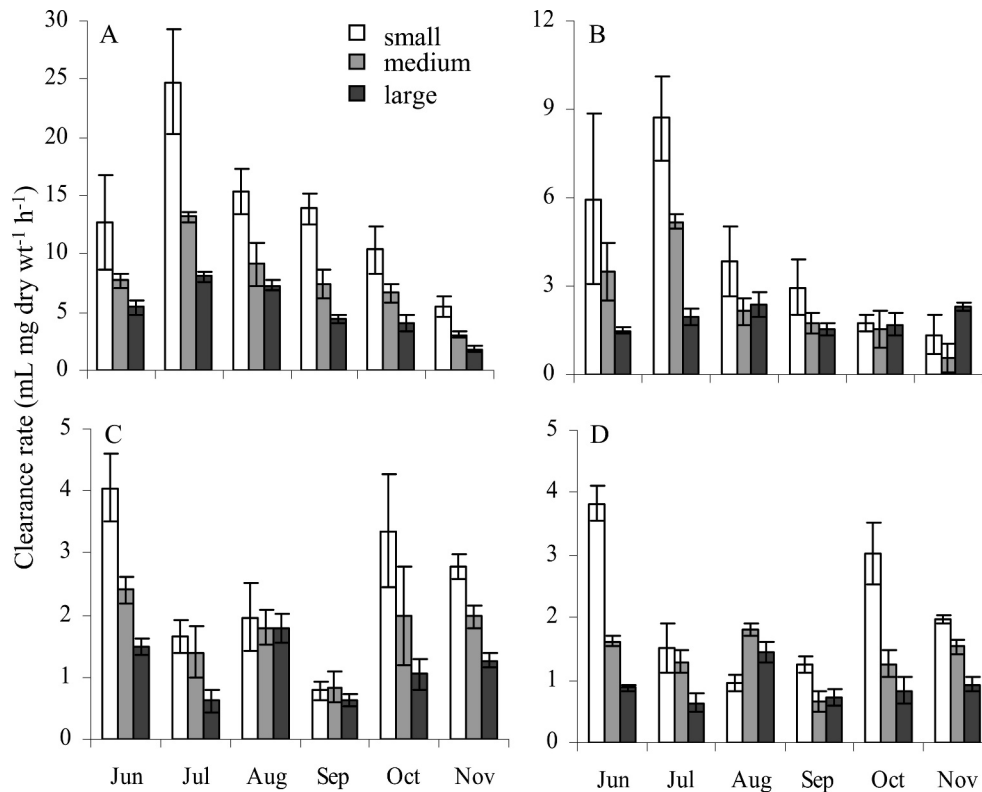


Fig. 1. Clearance rate (mL mg dry wt<sup>-1</sup> h<sup>-1</sup>) of zebra mussels of (A) Chl *a*, (B) particulate phosphorus, (C) particulate nitrogen, and (D) particulate carbon. Mean values ( $\pm 1$  SE;  $n = 3$  per bar) are given.

Table 3. Two-way ANOVAs for comparisons of NO<sub>3</sub><sup>-</sup>NO<sub>2</sub><sup>-</sup>-N, PO<sub>4</sub><sup>3-</sup>-P, NH<sub>4</sub><sup>+</sup>-N, total phosphorus (TP), and total nitrogen (TN) concentrations and dissolved N:P ratio in treatment vessels that were measured at the end of each experiment. Differences among the treatment are indicated by different superscript lowercase letters.

Dependent variable	Independent variable	df	<i>F</i>	Tukey test results*
NO <sub>3</sub> <sup>-</sup> NO <sub>2</sub> <sup>-</sup> -N	Month	5	1,096.2 (<0.001)	
	Treatment	3	1.6 (0.2)	
	Interaction	15	2.0 (<0.05)	
	Error	48		
PO <sub>4</sub> <sup>3-</sup> -P	Month	5	772.3 (<0.001)	
	Treatment	3	41.9 (<0.001)	Control <sup>a</sup> ; small <sup>b</sup> ; medium <sup>b</sup> ; large <sup>c</sup>
	Interaction	15	7.2 (<0.001)	
	Error	48		
NH <sub>4</sub> <sup>+</sup> -N	Month	5	121.6 (<0.001)	
	Treatment	3	153.0 (<0.001)	Control <sup>a</sup> ; small <sup>b</sup> ; medium <sup>c</sup> ; large <sup>d</sup>
	Interaction	15	7.8 (<0.001)	
	Error	48		
TP	Month	5	14.1 (<0.001)	
	Treatment	3	1.2 (0.3)	
	Interaction	15	0.5 (0.9)	
	Error	48		
TN	Month	5	17.9 (<0.001)	
	Treatment	3	1.2 (0.3)	
	Interaction	15	0.4 (1.0)	
	Error	48		
Dissolved N:P ratio	Month	5	1,110.9 (<0.001)	
	Treatment	3	16.7 (<0.001)	Control <sup>a</sup> ; small <sup>b</sup> ; medium <sup>bc</sup> ; large <sup>c</sup>
	Interaction	15	3.4 (<0.01)	
	Error	48		

Table 4. Two-way ANOVAs for comparisons of molar C:N:P ratios of seston stoichiometry in treatment vessels measured at the end of each experiment. Differences among the treatment are indicated by different superscript lowercase letters.

Dependent variable	Independent variable	df	F	Tukey test results*
Seston C:P ratio	Month	5	22.6 (<0.001)	
	Treatment	3	9.6 (<0.001)	Control <sup>a</sup> ; small <sup>b</sup> ; medium <sup>b</sup> ; large <sup>b</sup>
	Interaction	15	3.4 (<0.01)	
	Error	48		
Seston N:P ratio	Month	5	22.4 (<0.001)	
	Treatment	3	4.0 (<0.05)	Control <sup>a</sup> ; small <sup>b</sup> ; medium <sup>b</sup> ; large <sup>b</sup>
	Interaction	15	3.1 (<0.01)	
	Error	48		
Seston C:N ratio	Month	5	26.6 (<0.001)	
	Treatment	3	7.3 (<0.001)	Control <sup>a</sup> ; small <sup>a</sup> ; medium <sup>b</sup> ; large <sup>b</sup>
	Interaction	15	2.2 (<0.05)	
	Error	48		

\* Tukey test results were shown only for "treatment" variable.

of C:N:P of the seston at the end of the experiment. The mussels increased the molar ratios of C:P and N:P during June through August and caused them to decline in October and November (Fig. 2A,B).

The change in C:P and N:P ratios of seston by zebra mussels varied among months and size-classes (Table 5). The mean changing rate of elemental ratios of seston by zebra mussels ranged from  $-0.88$  to  $+4.67$  mg dry wt<sup>-1</sup> d<sup>-1</sup> for C:P and from  $-0.14$  to  $+0.47$  mg dry wt<sup>-1</sup> d<sup>-1</sup> for N:P among size-classes. The changing rates per day for C:P and N:P ratios of seston were significantly different from zero for all months (all  $t > 2.6$ ,  $df = 8$  month<sup>-1</sup>,  $p < 0.05$ ), with the exception of November, when the ratio for C:P did not differ from zero ( $t = -1.7$ ,  $df = 8$  month<sup>-1</sup>,  $p > 0.05$ ). There was a significant difference in changing rate of C:P and N:P ratios of seston by zebra mussels between the June–July period and the October–November period (Table 5). Small mussels changed the molar ratios of C:P, and N:P for seston in higher rates per milligram dry weight than did mussels from the medium and large size-classes (Table 5).

Molar C:N ratio of seston increased significantly only in vessels with large mussels at the end of experiment (Table 4). The mean changing rate for C:N ratio of seston varied between  $-0.01$  and  $+0.02$  mg dry wt<sup>-1</sup> d<sup>-1</sup> among size-classes and did not differ from zero during the July through November period (all  $t < 2.1$ ,  $df = 8$  month<sup>-1</sup>,  $p > 0.05$ ; Fig. 3) but not in June ( $t = 4.4$ ,  $df = 8$  month<sup>-1</sup>,  $p < 0.05$ ; Fig. 3), when the mussels increased the C:N ratio of seston. The size had no effect on changing rate of C:N ratio for seston per milligram dry weight of zebra mussels (Table 5).

*Changes in seston stoichiometry through nutrient excretion*—There was no significant difference in  $\text{NO}_3^-/\text{NO}_2^-$ -N concentration among treatments, indicating that the mussels did not excrete nitrate or nitrite ( $p > 0.05$ ; Table 3). Ammonium and soluble reactive phosphorus increased significantly at the end of experiment among treatments ( $p < 0.001$ ; Table 3; Fig. 3A,B), revealing excretion of some of the ingested seston in the form of soluble nutrients. In addition, the molar inorganic N:P

ratio changed significantly at the end of experiment among treatments ( $p < 0.001$ ; Table 3; Fig. 3C), indicating that mussel N:P excretion had an effect on seston dissolved N:P ratio. The value of inorganic N:P ratio of seston was increased across the months, except in August, when the N:P ratio of seston declined (Fig. 3C).

Ammonium and phosphate concentrations were significantly higher in vessels with large mussels than in those with small and medium mussels after the 6-h experiment (Table 3), indicating that large mussels excreted more nutrients per individual than did mussels from small and medium size-classes (Table 3; Fig. 3A,B). However, small mussels excreted more nutrients per milligram dry weight than did other size-classes (Table 5; Fig. 4).

The excretion rates of soluble nutrients and inorganic N:P ratio by zebra mussel varied over time (Table 5). The phosphate excretion rate was relatively high in July (Table 5; Fig. 4, left panels). The ammonium excretion rate of zebra mussels was high during June through August (temperature between  $17.8^\circ\text{C}$  and  $19.1^\circ\text{C}$ ; Table 1) and low during September through November (temperature between  $4^\circ\text{C}$  and  $14^\circ\text{C}$ ; Table 1) (Table 5; Fig. 4, right panels). Small mussels excreted at a significantly lower N:P ratio than did medium and large mussels (Table 5). Over all months, the excretion rates of molar N:P ratios (mean N:P [ $\pm 1$  standard error (SE)],  $n = 18$ ) was  $14.6:1 \pm 2.2$  for small,  $20.7:1 \pm 3.7$  for medium, and  $20.3:1 \pm 3.2$  for large size-classes of mussels.

*Nutrient uptake vs. nutrient excretion*—Uptake rate of particle nutrients and excretion rate of soluble nutrients were significantly different from zero for all months in Lake Erken (Fig. 4; all  $t > 3.3$ ,  $df = 8$  month<sup>-1</sup>,  $p < 0.05$ ). When experiments from all months were combined, the estimated nutrient budget showed a significantly higher uptake rate of particle nutrients than regeneration of soluble nutrients by zebra mussels (one-way ANOVA,  $F_{1,106} = 34.4$ ,  $p < 0.001$  for P; one-way ANOVA,  $F_{1,106} = 11.2$ ,  $p < 0.001$  for N; Fig. 4). Monthly estimated nutrient budgets indicated no significant difference between the uptake of PP and the release of  $\text{PO}_4^{3-}$ -P by the mussels in June (one-way ANOVA,  $F_{1,16} = 4.0$ ,  $p > 0.05$ ), September

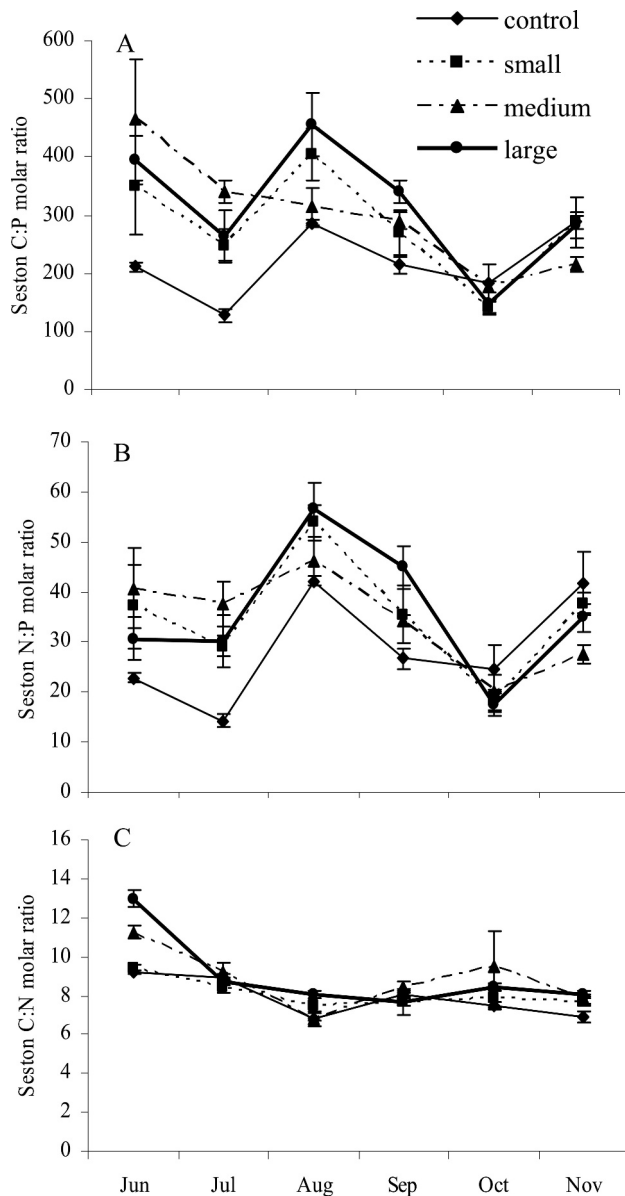


Fig. 2. Molar ratios of C:P, N:P, and C:N of seston stoichiometry in treatment vessels that were measured at the end of each experiment (after 6 h) from June to November. Mean values ( $\pm 1$  SE;  $n = 3$  error bar<sup>-1</sup>) are given.

( $F_{1,16} = 3.4$ ,  $p > 0.05$ ), October ( $F_{1,16} = 2.3$ ,  $p > 0.05$ ), and November ( $F_{1,16} = 0.1$ ,  $p > 0.05$ ). In addition, there was no significant difference between the uptake rate of PN and release of  $\text{NH}_4^+$ -N by the mussels in June ( $F_{1,16} = 2.4$ ,  $p > 0.05$ ).

Size and month  $\times$  size interaction had no effect on the ratio of nutrient release to nutrient uptake rates by zebra mussels ( $p > 0.05$ ), whereas there was a significant effect of month on both ratios of P release to P uptake (two-way ANOVA,  $F_{5,36} = 7.4$ ,  $p < 0.001$ ) and N release to N uptake (two-way ANOVA,  $F_{5,36} = 16.7$ ,  $p < 0.001$ ). Overall for all size-classes, the monthly ratios were 0.42, 0.28, 0.28, 0.58, 0.84, and 0.57 for P release to P uptake and 0.66, 2.26, 0.7, 0.69, 0.34, and 0.33 for N release to N uptake from June to

November, respectively. The ratios of P release to P uptake in June through August were significantly lower than in October through November (Tukey test,  $p < 0.05$ ). This ratio in September did not differ from other months (Tukey test,  $p > 0.05$ ). In addition, a Tukey test revealed that the ratio of N release to N uptake rate by mussels in July differed from that of other months ( $p < 0.05$ ).

*Tissue stoichiometry and nutrient budget of zebra mussels*—C:P, N:P, and C:N molar ratios of mussel body tissue varied significantly among months (Table 6; Fig. 5). The C:P and N:P ratios tended to be highest in August and September, whereas the C:N ratio was inclined to be highest in June. When all data were pooled, the molar ratios of C:P, N:P, and C:N were lower in small mussels than in the mussels from medium and large size-classes, although the effect of size on C:N:P stoichiometry of mussels was not significant (Table 6).

The turnover time of N and P in small mussels was shorter than that in medium and large size-classes of mussels (Fig. 6A,B). The turnover of P was highest in June (Fig. 6A), and the turnover of N was shorter during June through August than in September through November (Fig. 6B). On average, P turnover (63 d) was shorter than N turnover (127 d).

## Discussion

The results of this study showed that zebra mussels could alter the stoichiometry of seston through removal of particulate organic nutrients and could change the stoichiometry of the dissolved nutrient pool through nutrient excretion, supporting our hypothesis. We observed strongest effects of grazers on C:P and N:P ratios of seston, whereas the C:N ratios were less affected or unaffected, corroborating earlier findings from pelagic communities (Urabe 1995) and benthic communities (Hillebrand and Kahlert 2001). In our experiment ammonium was the main component of the DIN, congruent with most observations on benthic nitrogen regeneration (Prins and Small 1994; Gardner et al. 1995). Conversely, the oyster (*Crassostrea gigas*) is able to excrete the soluble N in the form of nitrate (Boucher and Boucher-Rodoni 1988). To provide valuable insight into zebra mussel effects on nutrient dynamics and seston stoichiometry, we first discuss how seston stoichiometry is affected by zebra mussels' nutrient consumption and regeneration in combination with *Dreissena's* role in nutrient limitation and resource availability for phytoplankton growth. We then evaluate zebra mussels' significance in Lake Erken to translate our findings to the community level.

*Nutrient consumption*—Zebra mussels reduced P availability through enhancing C:P and N:P molar ratios of seston during June through August, when P was limited in the lake. This was probably a result of the relatively high clearance rate for PP and the low clearance rate for PN and PC, potentially leading to a P limitation for phytoplankton growth during this time period. Increased C:P and N:P ratios of seston can translate into low food quality (Hessen

Table 5. Two-way ANOVAs for the effects of month and size on the changing rate of molar ratios of C:N:P (ratio mg dry wt<sup>-1</sup> d<sup>-1</sup>) of seston stoichiometry and the excretion rate of soluble nutrients ( $\mu\text{g mg dry wt}^{-1} \text{d}^{-1}$ ) and inorganic N:P ratio by zebra mussels. For each row, similar superscript lowercase letters (a, b, and c) indicate groups that are not significantly different ( $p > 0.05$ , Tukey test).

Dependent variable	Independent variable	df	F	Tukey test results
C:P changing rate	Month	5	6.8 (<0.001)	Jun <sup>a</sup> ; Jul <sup>a</sup> ; Aug <sup>ab</sup> ; Sep <sup>ab</sup> ; Oct <sup>b</sup> ; Nov <sup>b</sup>
	Size	2	4.6 (<0.05)	Small <sup>a</sup> ; medium <sup>b</sup> ; large <sup>b</sup>
	Interaction	10	1.2 (0.29)	
	Error			
Organic N:P changing rate	Month	5	9.3 (<0.001)	Jun <sup>a</sup> ; Jul <sup>a</sup> ; Aug <sup>ab</sup> ; Sep <sup>ab</sup> ; Oct <sup>bc</sup> ; Nov <sup>c</sup>
	Size	2	6.3 (<0.01)	Small <sup>a</sup> ; medium <sup>b</sup> ; large <sup>c</sup>
	Interaction	10	1.7 (0.12)	
	Error			
C:N changing rate	Month	5	4.2 (<0.01)	Jun <sup>a</sup> ; Jul <sup>b</sup> ; Aug <sup>ab</sup> ; Sep <sup>b</sup> ; Oct <sup>ab</sup> ; Nov <sup>ab</sup>
	Size	2	1.2 (0.32)	
	Interaction	10	1.4 (0.24)	
	Error	36		
PO <sub>4</sub> <sup>3-</sup> -P excretion rate	Month	5	3.2 (<0.05)	Jun <sup>a</sup> ; Jul <sup>b</sup> ; Aug <sup>ab</sup> ; Sep <sup>ab</sup> ; Oct <sup>ab</sup> ; Nov <sup>ab</sup>
	Size	2	43.8 (<0.001)	Small <sup>a</sup> ; medium <sup>b</sup> ; large <sup>b</sup>
	Interaction	10	1.1 (0.41)	
	Error			
NH <sub>4</sub> <sup>+</sup> -N excretion rate	Month	5	74.0 (<0.001)	Jun <sup>a</sup> ; Jul <sup>a</sup> ; Aug <sup>a</sup> ; Sep <sup>b</sup> ; Oct <sup>b</sup> ; Nov <sup>b</sup>
	Size	2	67.8 (<0.001)	Small <sup>a</sup> ; medium <sup>b</sup> ; large <sup>c</sup>
	Interaction	10	3.2 (<0.01)	
	Error	36		
Inorganic N:P excretion rate	Month	5	27.7 (<0.001)	Jun <sup>a</sup> ; Jul <sup>a</sup> ; Aug <sup>a</sup> ; Sep <sup>b</sup> ; Oct <sup>b</sup> ; Nov <sup>b</sup>
	Size	2	3.9 (<0.05)	Small <sup>a</sup> ; medium <sup>b</sup> ; large <sup>b</sup>
	Interaction	10	1.7 (0.13)	
	Error	36		

1997; Elser et al. 2000a; Sterner and Elser 2002), which may in turn lead to declines in the energy transfer efficiency from primary producers to upper trophic levels in the pelagic food web (e.g., MacIsaac 1996). Conversely, the mussels increase P availability by decreasing C:P and N:P molar ratios of seston during October and November, when there was no limitation of P, possibly because of relatively high clearance rates for PN and PC and a low clearance rate for PP, although the mussel changing rate for C:P molar ratio did not differ from zero in November. Zebra mussels contributed to the N deficiency of the phytoplankton by increasing C:N molar ratio of seston only in June, when DIN was at the minimum level in the lake (Table 1). This supported our hypothesis that the flux in nutrients caused by differences in zebra mussel consumption would lead to a variation in phytoplankton nutrient limitation. The mean changing rate for C:N ratio of seston did not differ from zero during July through November. This can be explained by nonsignificant differences between zebra mussels' clearance rates for PN and for PC, although the clearance rate for PN was slightly higher than that for PC (Fig. 1).

The variation in the rate by which mussels cleared particular nutrients can be suggested to be related to the level of Chl a, because *Dreissena* grazed efficiently on phytoplankton (defined as Chl a), corresponding to the results of other studies (Fanslow et al. 1995; MacIsaac 1996; Naddafi et al. 2007b). The differences between the clearance rate of Chl a and that of PP can be explained by including P content of microzooplankton and other

organisms in our measured PP of seston. As a result, P was a better predictor of chlorophyll than was N or C in our study (Prairie et al. 1989). This may explain why the mussel clearance rate for PP is higher than that for other nutrients.

On the other hand, most of the POC is usually bound in detritus and in living organisms (Lampert and Sommer 1997). Detritus is the dominant sestonic fraction in many lakes (Hessen et al. 2003) and usually has a low nutritional value, because it is a product of the feeding activities of higher organisms and microorganisms (Lampert and Sommer 1997). Although many organisms cannot avoid eating detritus along with living food, zebra mussels have been shown to avoid low-quality food resources by rejecting these particles (Naddafi et al. 2007b). Similarly, a mussel (*Mytilus edulis*) bed has been shown to retain phytoplankton more efficiently than POC (Prins et al. 1996).

The clearance rates of different food types (Table 2) and the changing rate of C:P and N:P ratios of seston (Table 5) were different among mussel sizes. However, the different sizes of mussels did not lead to different effects on final ratios of C:P and N:P (see Table 4); they all tended to cause elevated C:P and N:P ratios, indicating a high net retention of P. Furthermore, there was a consistent effect of size on final ammonium concentration (Fig. 3B). This was probably due to the consistent N excretion and consumption rates by different size-classes among months (Fig. 4, right panels). However, there was no difference in tissue N:P or C:N ratios among size-classes of mussels

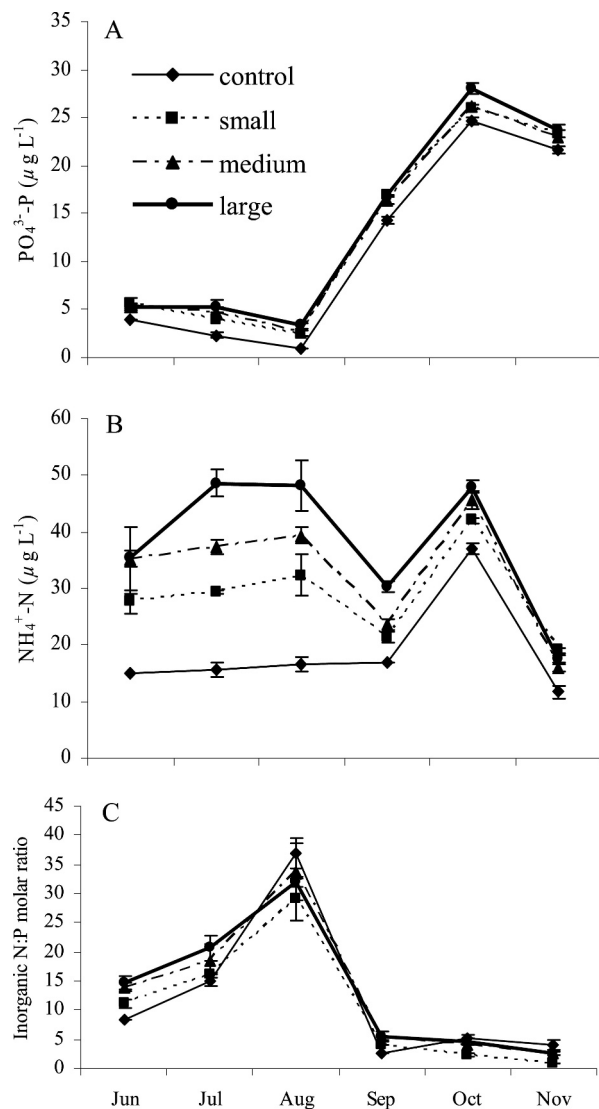


Fig. 3. Concentrations of (A)  $\text{PO}_4^{3-}\text{-P}$ , (B)  $\text{NH}_4^+\text{-N}$ , and (C) inorganic N:P ratio in treatment vessels that were measured at the end of each experiment (after 6 h) from June to November. Mean values ( $\pm 1$  SE;  $n = 3$  error bar $^{-1}$ ) are given.

sampled from one of the sites in this study, but we have observed in another study that zebra mussels from different depths and different sites in Lake Erken display a negative relationship between mussel size and tissue N:P ratio (R. Naddafi, K. Pettersson, and P. Eklöv unpubl.).

**Nutrient excretion**—The concentrations of nutrients in the dissolved phase were positively affected by the presence of mussels, indicating that the mussels increased dissolved nutrient availability by ammonium and phosphate excretion. Although we measured net rather than actual nutrient excretion rate by zebra mussels, our values correspond well with those of Arnott and Vanni (1996), but only the P excretion value was in agreement with that of Conroy et al. (2005). During June through August, the mean ammonium and phosphate excretion rate by mussels in our study ranged from 0.54 to 3.58 and from 0.06 to

0.57  $\mu\text{g mg dry wt}^{-1} \text{d}^{-1}$ , respectively (Fig. 4), compared to 0.59 to 3.53 and 0.09 to 0.65  $\mu\text{g mg dry wt}^{-1} \text{d}^{-1}$  in the Arnott and Vanni (1996) study.

Furthermore, we found that the mass-specific nutrient excretion rate of zebra mussels (see Fig. 4) was higher for small size-classes than for medium and large size-classes of mussels. This could be explained by allometric constraints on metabolism (Peters 1983). A decline of nutrient excretion with increasing body mass has been observed in many field studies (Vanni 2002). Conversely, Conroy et al. (2005) found that nutrient excretion of zebra mussels collected from the western basin of Lake Erie increased with increasing body mass. Some of these results can be explained by the fact that the measurements were done in July, right after the major release of gametes in the summer. Large mussels would presumably be more physiologically affected by this metabolically expensive activity and then may have been “leaking” nutrients. However, Arnott and Vanni (1996) found the trend of increasing nutrient concentration with increasing body mass in July only for ammonium.

In the present study, the dissolved inorganic N:P molar ratio of seston was reduced by zebra mussel presence largely in August. However, a small decrease in dissolved N:P ratio by zebra mussels was also observed in fall. The direction of changes in dissolved nutrients can be under the control of both consumer body stoichiometry (Vanni et al. 2002) and food nutrient composition (Elser and Urabe 1999). Based on ecological stoichiometry theory, an animal with a relatively low P content (i.e., high N:P ratio) in its body tissue seems to allocate less P to growth and will probably excrete more P than will an animal with a high body P content. Thus, the N:P ratio excreted by an animal should be negatively correlated with the N:P of its body tissues (Vanni 2002). Consequently, it is possible that the skewed N:P ratio of seston in August reflects a similar pattern in our study (see Fig. 5B), although we do not know whether or not this is related to a higher accumulation of N in zebra mussel tissue (see below and Fig. 6B) and a homeostatic excretion by the mussels.

**Tissue stoichiometry and nutrient budget of zebra mussels**—The current study indicated higher C:P and N:P molar ratios for zebra mussel tissue in August, and to some degree in September, than in all other months; this may be because of a decrease in soft tissue P content during the summer as a result of gametogenesis, as has been observed in zebra mussels in Polish lakes (Stańczykowska and Planter 1985) and in western Lake Erie (Arnott and Vanni 1996). Further, the variation in C:N:P stoichiometry for zebra mussels over time or space can be explained by rheostasis, as suggested by Villar-Argaiz et al. (2002), who implied that the nutrient stoichiometry of organisms appears to change as a function of the environment they inhabit.

The tissue C:P ratio (see Fig. 5A) in this study corresponds well with that described by Liess and Hillebrand (2005) for the zebra mussel. Such a value of the C:P ratio has been shown to be higher than the tissue C:P ratio of most of the benthic invertebrates in the littoral zone of Lake Erken, including Gastropoda, Trichoptera,

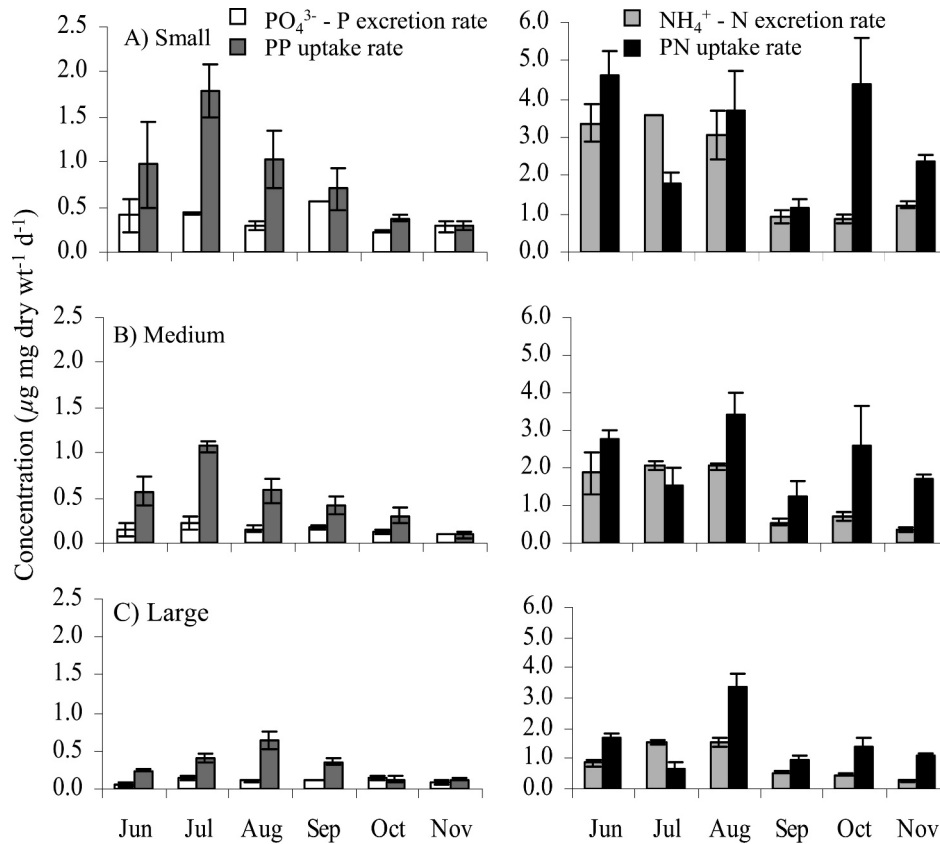


Fig. 4. Soluble phosphate ( $\text{PO}_4^{3-}\text{-P}$ ) excretion rate and particulate phosphorus (PP) uptake rate (left panels) and soluble ammonium ( $\text{NH}_4^+\text{-N}$ ) excretion rate and particulate phosphorus (PN) uptake rate (right panels) of (A) small, (B) medium, and (C) large size-classes of zebra mussels in Lake Erken. Mean values ( $\pm 1$  SE;  $n = 3$  per bar) are given.

Diptera, Ephemeroptera, Isopoda, and Hydracarina (Lies and Hillebrand 2005). Hence, *Dreissena* appears to be less vulnerable to P limitation. This may in turn explain to some degree the invasion success of this species. However, when this species invades the novel ecosystems, it may increase P limitation through increasing sestonic C:P ratio and high P retention.

We found that the turnover times of P and N relative to tissue content were shorter in smaller mussels, indicating

that loss of elements through excretion was relatively high in comparison with tissue content. This, in conjunction with high mass-specific nutrient excretion by small mussels, indicates that a community consisting of small consumers is a more efficient recycler than is a community comprising large consumers of the same biomass; this result corresponds to those of other studies (Vanni et al. 2002). This may be especially important in lakes in which the vast share of the zebra mussel population is small (e.g., western Lake

Table 6. Two-way ANOVAs for the effect of month and size on the molar C:N:P ratios of the mussel tissue. Differences among the month are indicated by different superscript lowercase letters.

Dependent variable	Independent variable	df	F	Tukey test results
Tissue C:P ratio	Month	5	7.3 (<0.001)	Jun <sup>ab</sup> ; Jul <sup>b</sup> ; Aug <sup>c</sup> ; Sep <sup>ac</sup> ; Oct <sup>ab</sup> ; Nov <sup>ab</sup>
	Size	2	2.1 (0.1)	
	Interaction	10	0.9 (0.5)	
	Error	216		
Tissue N:P ratio	Month	5	6.9 (<0.001)	Jun <sup>a</sup> ; Jul <sup>ab</sup> ; Aug <sup>c</sup> ; Sep <sup>bc</sup> ; Oct <sup>abc</sup> ; Nov <sup>ab</sup>
	Size	2	2.2 (0.1)	
	Interaction	10	1.3 (0.2)	
	Error	216		
Tissue C:N ratio	Month	5	44.9	Jun <sup>a</sup> ; Jul <sup>b</sup> ; Aug <sup>c</sup> ; Sep <sup>c</sup> ; Oct <sup>b</sup> ; Nov <sup>c</sup>
	Size	2	0.2 (0.8)	
	Interaction	10	3.6 (<0.001)	
	Error	216		

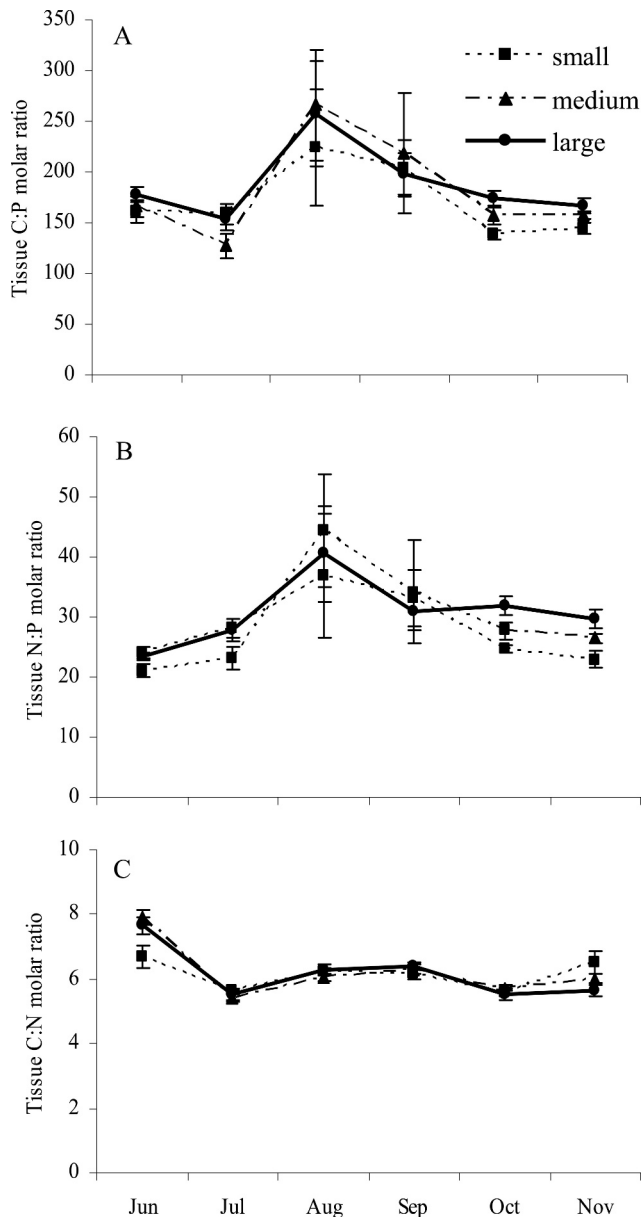


Fig. 5. Molar ratios of (A) C:P, (B) N:P, and (C) C:N for zebra mussels from June to November. Mean values ( $\pm 1$  SE;  $n = 15$  error bar $^{-1}$ ) are given.

Erie in 1990; Bunt et al. 1993), particularly after the reproduction season. On the other hand, small mussels grow more than large ones, and they may therefore have a larger effect on the N:P ratio of lakes. In addition, growth will also remove nutrients from the water, and faster feeding rates may produce more feces and pseudo-feces and thus increase sedimentation rates. This can in turn affect the relative importance of TP and soluble reactive P in the ecosystem (see below).

During June through August the turnover time for N (see Fig. 6A) was shorter than during the September–November period, indicating that zebra mussels provide more ammonium in summer. But the turnover time of P (see Fig. 6B) was longer in June, when P was at the lowest

level in the lake (see Table 1), revealing that *Dreissena* increase P limitation when P is limiting through absorbing P. This latter result indicates that the consumer will store P and release it at low rates when the concentration is low in the water. This will in turn result in longer turnover of P. Furthermore, longer turnover time of N than P indicates that N was retained more efficiently than P in *Dreissena* tissue, which is similar to what Smaal and Vonck (1997) found in *Mytilus edulis* (but see Hatcher 1994). Since the N:P ratio of seston was generally lower and the C:N was higher in the mussel tissue, it appears that N availability in food is relatively limited. This, together with increased retention of more N than P in *Dreissena* biomass, may contribute to the prevalence of algal N rather than P deficiency in lakes such as Lake Erken, thereby resulting in higher success of  $N_2$ -fixing cyanobacteria in the ecosystem. For instance, the occurrence of *Gloeotrichia echinulata* blooms, which have been reported since the early 1900s in Lake Erken (K. Pettersson unpubl.), increased after zebra mussel invasion (K. Pettersson unpubl.). Such dominance of cyanobacteria may cause low trophic transfer efficiency (Hessen et al. 2005).

However, increased sestonic C:P and N:P ratios during summer are thought to decrease cyanobacteria in Lake Erken. But this may constrain the growth of non-nitrogen-fixing cyanobacteria such as *Microcystis*, which is a slow-growing species with high optimal temperature (Bastviken et al. 1998). The high buoyancy rates of *G. echinulata* coupled with unique P uptake and its specific life strategy, which comprises both pelagic and benthic stages, result in a competitive advantage in Lake Erken as a result of the lake's high rates of light absorption and higher growth rates (Rodrigo et al. 1998; Karlsson-Elfgren et al. 2004). Alternatively, decreased sestonic C:P and N:P ratios may favor cyanobacteria, including *Microcystis*, in fall, when *G. echinulata* is deposited on the sediment in the form of resting cells (Karlsson-Elfgren et al. 2004). Although *Microcystis* is relatively abundant in fall compared to other seasons, it does not bloom in Lake Erken, presumably as a result of the lake's low temperature (K. Pettersson unpubl.).

*Zebra mussel significance in Lake Erken*—A study of size structure of the mussel population in Lake Erken in 2005 showed that small, medium, and large size-classes constitute 46%, 35%, and 19% of the zebra mussel population, respectively (R. Naddafi unpubl.). In addition, zebra mussel lake-wide biomass calculated based on areas occupied and unoccupied by the mussels revealed that biomasses of small, medium, and large size-classes of zebra mussels were 7.3, 5.4, and 3.0 g dry wt  $m^{-2}$ , respectively, (15.7 g dry wt  $m^{-2}$  in total) in Lake Erken in 2005 (R. Naddafi unpubl.). Given a mean chlorophyll clearance rate (Fig. 1A), the zebra mussel population in Lake Erken filtered 4.4  $m^3 m^{-2} d^{-1}$  in 2005. With water volume of the Lake Erken estimated at  $213.5 \times 10^6 m^3$  (K. Pettersson unpubl.), the mussel population was theoretically capable of filtering the entire volume of Lake Erken 0.2 times per day in 2005 during the June through November period. Such filtration capacity of zebra mussels in Lake Erken is

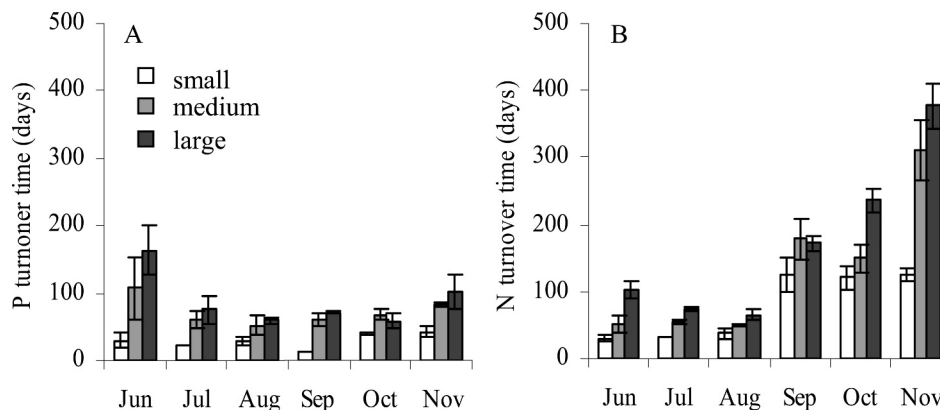


Fig. 6. Turnover time per (A) phosphorus and (B) nitrogen per season. Mean values ( $\pm 1$  SE;  $n = 3$  per bar) are given.

comparable with that of European and some North American lakes (R. Naddafi unpubl.). However, it is possible that we have overestimated the realized mussel feeding rates in Lake Erken because of the static experimental conditions in the enclosures. In the lake, physical dynamics will be important in terms of how available phytoplankton is to bottom-dwelling organisms such as mussels (Ackerman 1999; Ackerman et al. 2001).

Furthermore, based on *Dreissena* mean nutrient excretion rate (Fig. 4A–C), the zebra mussel population could release 3.9 mg phosphate  $m^{-2} d^{-1}$  and 25.3 mg ammonium  $m^{-2} d^{-1}$  in 2005. Zebra mussel standing stock could release 37 kg soluble reactive P and 240 kg ammonium per day into the water column of Lake Erken. Finally, zebra mussel P recycling of 6,660 kg during June through November exceeds the Malmaeus and Rydin (2006) estimates of annual P release of 3,000 kg from the profundal sediments of Lake Erken. Nutrient excretion by *Dreissena* can be greater than many sources of nutrient input to lakes. For example, P flux from zebra mussels has been suggested to exceed P release from sediments, P excretion by other animals, macrophytes' P release, and external P loading (Arnott and Vanni 1996). However, zebra mussels feed mainly on plankton and are more likely to recycle rather than changing the total amount of nutrients (Vanni 2002). Sediments may release stored P, which can be available for primary production. Thus, P released from the sediments can increase the TP concentration in the lakes, but the recycling of available P by mussels should mainly decrease the TP in the water column. For example, TP decreased slightly, but not significantly ( $p > 0.05$ ; see Table 3; but see James et al. 1997), at the end of our experiments.

*Nutrient uptake vs. nutrient excretion*—Zebra mussels can act either as nutrient sinks or nutrient sources in lakes, but the direction of the flux of nutrients depends mainly on zebra mussel population density and biomass (Heath et al. 1995; Mellina et al. 1995; James et al. 1997). We found that nutrient mass-specific uptake rate is higher than excretion rate by zebra mussels. Thus, dense zebra mussel populations with high biomass can sequester a large amount of nutrients in their tissue over relatively short time scales and can thereby act as nutrient sinks in lakes (Johengen et al.

1995); this function has also been observed in the P budget of *Daphnia* (Urabe et al. 1995). In fact, Mellina et al. (1995) found that the grazing pressure of zebra mussels exceeded phytoplankton growth in Lakes Erie and St. Clair, in which the mussels were able to decouple the nutrient–chlorophyll relationship, as well as in the inner portion of Saginaw Bay, Lake Huron (Johengen et al. 1995), but not in Oneida Lake and European lakes (Mellina et al. 1995). For example, Fanslow et al. (1995) demonstrated that filtering activities of the mussel population can account for the decline in chlorophyll and the increase in water clarity. Hence, although the relative effect of filtration and nutrient generation on phytoplankton dynamics is poorly understood (Conroy et al. 2005), it is possible that zebra mussels function as a sink rather than a source for nutrients in lakes (Heath et al. 1995; James et al. 1997). However, nonlethal effects of predators can mediate zebra mussel clearance rate in the field, thereby cascading positive indirect effects on phytoplankton resources (Naddafi et al. 2007a). Appropriate long-term and controlled field experiments are needed to determine how nutrient excretion, nutrient consumption, and nonlethal effects of predators interact with each other to affect phytoplankton dynamics in the aquatic ecosystems.

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*Received: 7 November 2007*

*Accepted: 29 April 2008*

*Amended: 24 April 2008*