Spatial and seasonal variation in nutrient excretion by benthic invertebrates in a eutrophic reservoir

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SUMMARY
1. Nitrogen (N) and phosphorus (P) fluxes via excretion by benthic invertebrates were quantified in a eutrophic reservoir (Acton Lake, Ohio, U.S.A.). We quantified variation in nutrient fluxes seasonally (June until November 1997), spatially (three sites) and among taxa (chironomids, tubificid oligochaetes and Chaoborus).
2. The three taxa differed in spatial distribution and contribution to nutrient fluxes. Tubificids were the most abundant taxon at two oxic sites (1.5 and 4 m depth), and were exceedingly rare at an anoxic, hypolimnetic site (8 m). Chironomids were abundant only at the shallowest oxic site. Chaoborus was the only abundant taxon at the anoxic site. Total benthic invertebrate biomass was greatest at the shallowest site and lowest at the anoxic, hypolimnetic site.
3. Mass-specific excretion rate \([\mu\text{mol NH}_4-\text{N or soluble reactive P (SRP) excreted mg dry mass}^{-1} \text{h}^{-1}]\) varied among experiments and was influenced by temperature. Differences among taxa were not significant. Thus, nutrient flux through benthic invertebrates was affected more by total invertebrate biomass and temperature than by species composition.
4. Fluxes of N and P via benthic invertebrate excretion (\(\mu\text{mol NH}_4-\text{N or SRP m}^{-2} \text{day}^{-1}\)) were greatest at the oxic sites, where fluxes were dominated by the excretion of tubificids and chironomids. The N and P fluxes at the anoxic site were much lower, and were dominated by excretion by Chaoborus. The ratio of N and P excreted by the benthic invertebrate assemblage varied seasonally and was lowest at the anoxic site.
5. Comparison with other measured inputs shows that excretion by benthic invertebrates could be an important source of nutrients, especially of P. However, the relative importance of nutrient excretion by the benthos varies greatly spatially and temporally.

Keywords: benthos, nutrient budget, nutrient excretion, nutrient flux, reservoir

Introduction
Fluxes of nutrients from sediment to water can play a significant role in lake nutrient dynamics, and can be especially important in shallow, eutrophic lakes (Boström et al., 1982; Nürnberg, 1987, 1988; Caraco, Cole & Likens, 1991). Nutrients can be released from sediments through a variety of mechanisms, including redox reactions and the activities of benthic animals. Digestion and subsequent excretion of nutrients by benthic invertebrates may play a large role in regulating nutrient release from sediments (Gardner et al., 1981, 1983; Fukuhara & Sakamoto, 1987; Fukuhara & Yasuda, 1989; Svensson, 1997).

The rate at which benthic invertebrates release nutrients, and the importance of this relative to other fluxes, will depend on many factors. Benthic invertebrate biomass and species composition may play large roles. Physico-chemical conditions may be important through a variety of direct and indirect mechanisms. For example, benthic invertebrate
abundance and species composition are greatly influenced by oxygen concentration (Jónasson, 1984). Temperature exerts a regulatory effect on the rate at which individuals process, and hence excrete, nutrients. Temperature, oxygen and pH will also affect the rate at which nutrients are released via other processes, especially redox reactions (Boström et al., 1982; Caraco et al., 1991). Biological interactions also can play an indirect role. For example, fish predation can affect the distribution and abundance of benthic invertebrates and thereby, indirectly affect nutrient flux through the benthos (Svensson, Bergman & Andersson, 1999).

Our study had two main objectives. The first was to assess the seasonal, spatial and interspecific variation in nutrient excretion rates of benthic invertebrates in a eutrophic reservoir. The second objective was to evaluate whether nutrient flux via benthic invertebrate excretion represents an important nutrient flux, compared with other nutrient fluxes. We took great care to obtain excretion rates reflective of those in the lake by minimising the time the animals were held without food after capture. In contrast, most previous studies measured rates over longer periods of time postcollection, which can result in underestimates of in-lake rates (for exceptions see Gardner et al., 1981, 1983; Tuominen et al., 1999; Wilhelm, Hudson & Schindler, 1999).

Methods

Study site

Acton Lake is a 253 ha reservoir located in Hueston Woods State Park in south-western Ohio (mean depth 4 m; maximum depth ~8 m near the dam; Fig. 1). As in most reservoirs, ‘uplake’ areas of the lake (near stream inflows) are shallow and never

Fig. 1 Location and bathymetric map of Acton Lake. Depth contours are in metres. Sampling locations are Inflow site (I), Middle site (M) and Dam site (D). Inflow streams are denoted by ‘S’.
thermally stratify. In contrast, deeper areas (near the 
dam) are thermally stratified in the summer (Winner, 
Strecker & Ingersoll, 1962). The lake is eutrophic, 
receiving substantial inputs of nutrients from its 
large and primarily agricultural catchment (Winner 
et al., 1962; Daniel, 1972; Schaus et al., 1997; Vanni 
et al., 2001). Thus, hypolimnetic water is anoxic 
throughout summer (Winner et al., 1962; Evarts, 
1997). Previous and ongoing studies have quantified 
nutrient inputs to the water column from the catchment 
(Vanni et al., 2001), direct nutrient release from anoxic 
and oxic sediments (Evarts, 1997), and excretion by 
sediment-feeding fish (Mather et al., 1995; Schaus et al., 
1997). Therefore, we can compare our estimates of 
excretion by the benthos to these other fluxes. In 
addition, we can compare our data on benthic inverte-
brate abundance and species composition with those 
from earlier studies by Daniel (1972, 1984), who 
quantified the abundance of benthic invertebrates in 
Acton Lake from 1964 until 1976.

Benthic invertebrates were studied at three sites in 
Acton Lake (Fig. 1). The ‘Inflow’ site is approximately 
1.5 m deep, and remains isothermal and well-oxygen-
ated year-round. The ‘Middle’ site is approximately 
4 m deep. It is usually isothermal and well-oxygen-
ated even in summer, but occasionally becomes 
anoxic near the bottom during calm weather. The 
‘Dam’ site, approximately 8 m deep, is strongly 
stratified and is anoxic below 4 m from late May 
until late September (Winner et al., 1962; Evarts, 1997). 
Sediments at all three sites are comprised of finely 
divided clays, minerals and organic matter (‘gyttja’), 
although relatively coarse allochthonous materials 
(leaves, sticks) were often observed in sediments at 
the Inflow site.

Benthic invertebrate abundance and species 
composition

Benthic samples were collected with an Ekman grab 
with an area of 0.0289 m². Samples were taken on 10 
dates from early June until early November 1997. Ten 
grabs were taken from each site, with two neighbour-
ning grabs pooled together as one sample, yielding five 
replicate samples per site per date, with each replicate 
representing 0.0578 m² of sediment. Samples were 
passed through a sieve (~500 μm mesh) in the field to 
remove most sediments, and the remaining detritus 
and organisms washed into plastic cups with 95% 
ethanol. Invertebrates were sorted and counted using 
a dissecting microscope.

To obtain individual biomass estimates, length–mass 
regressions were developed for each of the 
three most common taxa (chironomids, tubificids and 
Chaoborus). On 10 October 1997, organisms were 
collected from the Inflow site and returned to the 
laboratory. Chironomids, tubificids and Chaoborus (30 
of each taxon) were picked at random, measured to 
the nearest 0.01 mm, dried overnight at 60 °C and 
weighed to the nearest microgram. The length–mass 
regressions developed for each taxon on this date 
were used to estimate biomass on all dates. To 
estimate population biomass on each sampling date, 
the length of 30 organisms of each of the three taxa 
was measured. Usually 10 individuals were measured 
at each site unless a taxon was not found at a 
particular site, in which case more individuals were 
measured at other sites to obtain a total of 30. The dry 
mass of each measured animal was calculated using 
the length–mass regression equations, and mean dry 
mass obtained for each taxon at each site. Mean dry 
mass (mg individual⁻¹) was multiplied by popula-
tion density (ind m⁻²) to generate biomass (mg dry 
mass m⁻²) at each site for the three taxa.

Statistical differences in population density among 
sites and dates, and site*date interactions, were 
assessed for each taxon using repeated measures 
ANOVA. Data were log(n + 1)-transformed to normal-
ise variance. Because oligochaetes were not quantified 
on the first sampling date, repeated measure ANOVAS 
were conducted without this date for all taxa. Because 
repeated measures ANOVA revealed significant site 
and date effects for all taxa, one-way ANOVA followed 
by Tukey’s test was performed on each date for each 
taxon to test for significant differences between sites. 
We did not analyse biomass data with ANOVA 
because biomass on a given date was obtained by 
multiplying density times a constant (mean individual 
biomass on that date); therefore all variation in 
biomass within a date was attributable to variation 
in density.

Nutrient excretion rates

Excretion experiments were conducted on six dates in 
1997: 17 June, 24 June, 17 July, 19 October and 9 
November. Temperatures ranged from 9 to 27 °C. We 
quantified excretion rates of nitrogen (as NH₄-N) and
phosphorus (as soluble reactive P, SRP). Three taxa (chironomids, tubificids and Chaoborus) were the most abundant throughout the study, as in earlier studies in Acton Lake (Daniel, 1972, 1984). Therefore, these taxa were used for excretion experiments. To conduct experiments, sediments containing animals were collected with a 0.0289 m² Ekman grab and washed through a sieve (~500 µm mesh) to remove sediments. Collection of each taxon for experiments reflected its relative abundance in the lake. Thus, chironomids were always taken from the Inflow site; Chaoborus was always taken at the Middle site (except on the first date, when larvae were taken from the Inflow site); oligochaetes were taken from both the Middle and Inflow sites. The sieve was then placed in a bucket with water, and animals were gently picked out with forceps and placed in enamel pans. This allowed the separation of detritus particles and algae from the organisms, as these particles could affect nutrient analyses. Organisms remained in the pans for <15 min. Once a sufficient number were found, all were transferred at the same time into 125 mL Nalgene bottles. Each replicate bottle was filled with lake water previously collected from well-oxygenated surface waters and filtered (Gelman A/E glass fibre filters, Pall Corporation, Ann Arbor, MI, USA) to remove particles that could absorb excreted nutrients. Each replicate contained up to 25 individuals of a single taxon. Bottles were sealed, placed in a net with a weight, and sent to the bottom of the lake, at the respective collection site. Up to five replicates of each taxon were used, depending on organism availability. The volume of water per bottle was 125 mL for the first experiment and 100 mL for all subsequent experiments. We decreased the volume to increase nutrient concentrations, thus facilitating NH₄–N and SRP analyses.

Organisms were incubated for 1 h when water temperature was ≥15 °C, and for 4 h when temperature was <15 °C. Short incubation times are essential for obtaining rates reflecting those in the lake. This is because excretion rate declines rapidly after animals are separated from their food, as has been shown for numerous aquatic animals, including zooplankton (Lehman, 1980; Gardner & Scavia, 1981), benthic invertebrates (Johannes, 1964; Gardner et al., 1981, 1983; Arnott & Vanni, 1996) and fish (Mather et al., 1995).

At the end of each experiment, water from each bottle was immediately filtered on site through preweighed Gelman A/E glass fibre filters. Samples of filtrate were stored at 4 °C until analysis, and then analysed manually for NH₄–N using the phenol-hypochlorite technique (Solorzano, 1969) and for SRP using the molybdenum blue technique (Murphy & Riley, 1962). Most chemical analyses were completed within 48 h, and all were completed within 1 week of collection. We verified that this storage period does not affect nutrient concentrations. Filters containing benthic invertebrates were dried overnight in an oven and then re-weighed to obtain dry mass of organisms (excepting the first two excretion experiments when we obtained dry masses using mean organism length at the site sampled for the experiment and the length–mass regression equations described above. This was performed because the filters were accidentally destroyed in the drying oven). Mass-specific excretion rates (N or P excreted mg dry mass⁻¹ h⁻¹) were calculated as final minus initial nutrient mass divided by dry mass of organisms. We also employed control bottles, without organisms, to verify that changes in concentration were not caused by exchange between bottle surfaces and water.

For some excretion experiments, less than five replicates were employed for one or more taxa. Because this led to an unbalanced design, repeated measures ANOVA could not be performed on excretion rates. Therefore, one-way ANOVA and Tukey’s test were performed separately on each date to test for interspecific differences in mass-specific N and P excretion rates and the N : P ratio excreted. Excretion rate data were log(x + 1) transformed prior to analyses.

**Nutrient flux rates**

Using data from excretion experiments, we generated regressions of mass-specific excretion rate versus temperature for each of the three taxa. We then used site-specific biomass and temperature data (water temperature just above the sediments) to obtain a nutrient flux rate for each taxon at each site on each date on which we quantified biomass. To do so, we multiplied mass-specific excretion rate (µmol N or P mg dry mass⁻¹ day⁻¹) times biomass at that site (mg dry mass m⁻²) to obtain a flux rate for that taxon (µmol N or P m⁻² day⁻¹). We also obtained site-specific fluxes for the entire assemblage by summing the flux rates of the three taxa at each site. To obtain lake-wide
flux rates, we assumed that flux rates at the Dam site were representative of anoxic sediments, while rates at the Inflow and Middle sites were representative of oxic sediments. In Acton Lake, anoxic and oxic sediments comprise roughly equal areas. Therefore, to obtain lake-wide rates we first averaged the Inflow and Middle site rates and then averaged this with Dam site rates. The ratio at which N : P was excreted by the entire assemblage (hereafter N : P excretion ratio) was also obtained. To obtain the N : P excretion ratio at a particular site, N flux (sum of the three taxa) at that site was divided by P flux (sum of the three taxa) at that site. To obtain a lake-wide estimate of N : P excretion ratio, the lake-wide N flux was divided by the lake-wide P flux.

Results

Benthic invertebrate densities

Chironomids, Chaoborus punctipennis (Say) and tubificid oligochaetes comprised the vast majority of benthic invertebrates in our samples. Chironomids were represented by several taxa, including large red forms (most likely of the subfamily Chironomini) and smaller brown forms (most likely of the subfamily Tanytarsini). The tubificids were most likely Limnodrilus hoffmeisteri (Claparede) (Daniel, 1972). Population densities of all three taxa showed significant differences across sites and dates (P < 0.0001; Table 1, Fig. 2). There was a significant site*date interaction for Chaoborus and chironomids; this interaction term was marginally significant for tubificids (Table 1). Length–mass regressions were similar for Chaoborus and chironomids, but the regression for tubificids was quite different from the dipteran taxa (Table 2). All taxa showed a tendency to increase in density and biomass during the study period; this trend was most pronounced for chironomids at the Inflow site and for Chaoborus at the Middle and Dam sites (Fig. 2).

For all taxa, densities were significantly different (P < 0.05) among sites on all dates. On all but two sampling dates, Chaoborus density was significantly lower at the Inflow site than the other two sites, and on most dates there was no significant difference in Chaoborus density between the Dam and Middle site (Fig. 2). Chaoborus was the only abundant taxon at the anoxic Dam site (Fig. 2). Chironomid density was significantly greater at the Inflow site than at the other two sites on all dates (Fig. 2). In addition, chironomids were significantly more abundant at the Middle site than the Dam site on all dates from 29 August onwards, although differences between these two sites were relatively small (Fig. 2). Tubificid density was significantly lower at the Dam site than the other two sites on all dates (Fig. 2). On the final two sampling dates, tubificid density was significantly higher at the Inflow site than at the Middle site; on other dates densities were not significantly different between these sites.

Mass-specific nutrient excretion rates

Mass-specific excretion rates were generally higher in June and July than in autumn, but were highly variable among dates, even within a taxon (Fig. 3). Overall, Chaoborus tended to have the highest, and chironomids the lowest, mass-specific P excretion rates (Fig. 3). However, there were no statistically significant differences among species on any date (P > 0.05). On 24 June and 17 July, there were marginally significant differences among taxa in N excretion rate (P = 0.060 and 0.067, respectively). In both cases, tubificids tended to excrete N at higher rates than the other taxa (Fig. 3). In addition, the ratio at which tubificids excreted N and P (N : P excretion ratio) was significantly greater than that of Chaoborus.

Table 1

<table>
<thead>
<tr>
<th>Data Source</th>
<th>d.f.</th>
<th>F</th>
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<td></td>
<td></td>
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<tr>
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<td>109.81</td>
<td>0.0001</td>
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<td>0.51</td>
<td>0.9193</td>
</tr>
<tr>
<td>Date</td>
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<td>48.26</td>
<td>0.0001</td>
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<td>Site*date</td>
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<td>4.5</td>
<td>0.0001</td>
</tr>
<tr>
<td>Chironomids</td>
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</tr>
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<td>301.05</td>
<td>0.0001</td>
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<td>1.3</td>
<td>0.1071</td>
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<td>18.66</td>
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<td>Site*date</td>
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<td>8.82</td>
<td>0.0001</td>
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<td>Oligochaetes</td>
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<td>Site*date</td>
<td>16</td>
<td>1.55</td>
<td>0.0992</td>
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</table>
There were no significant differences among taxa in N : P excretion ratio on other dates ($P > 0.05$). For all three taxa, mass-specific N excretion rate increased significantly with temperature (Table 2). For P excretion, the regression for Chaoborus was not significant, while those for chironomids and tubificids were significant. The percentage of variance in excretion rates explained by temperatures was relatively low ($\leq 33\%$ in all regressions; Table 2).

**Table 2** Regression equations relating length to mass, and mass-specific nutrient excretion rates to temperature. Units before transformation are mg for dry mass, mm for length, $\mu$mol N or P excreted mg dry mass$^{-1}$ h$^{-1}$ for excretion rates, and °C for temperature; $n$ refers to the number of individuals measured and weighed in the length–mass regressions, or to the number of bottles incubated to estimate excretion rates for the temperature-excretion rate regressions.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Regression equation</th>
<th>$P$</th>
<th>$r^2$</th>
<th>$n$</th>
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<tr>
<td>Chaoborus</td>
<td>Log dry mass = $-6.223 + 2.669 \times \log$ length</td>
<td>&lt;0.001</td>
<td>0.798</td>
<td>30</td>
</tr>
<tr>
<td>Chironomidae</td>
<td>Log dry mass = $-6.065 + 2.657 \times \log$ length</td>
<td>&lt;0.001</td>
<td>0.878</td>
<td>30</td>
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<tr>
<td>Tubificidae</td>
<td>Log dry mass = $-4.493 + 0.937 \times \log$ length</td>
<td>0.002</td>
<td>0.308</td>
<td>30</td>
</tr>
<tr>
<td>Chaoborus</td>
<td>Log(N excretion + 1) = $-0.0413 + 0.0441 \times \log$ temperature + 1</td>
<td>0.022</td>
<td>0.216</td>
<td>24</td>
</tr>
<tr>
<td>Chironomidae</td>
<td>Log(N excretion + 1) = $-0.0165 + 0.0188 \times \log$ temperature + 1</td>
<td>0.029</td>
<td>0.226</td>
<td>21</td>
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<tr>
<td>Tubificidae</td>
<td>Log(N excretion + 1) = $-0.0615 + 0.0611 \times \log$ temperature + 1</td>
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<td>0.328</td>
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<td>Log(P excretion + 1) = $-0.0030 + 0.0036 \times \log$ temperature + 1</td>
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<td>0.099</td>
<td>24</td>
</tr>
<tr>
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<td>Log(P excretion + 1) = $-0.0011 + 0.0012 \times \log$ temperature + 1</td>
<td>0.021</td>
<td>0.250</td>
<td>21</td>
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<tr>
<td>Tubificidae</td>
<td>Log(P excretion + 1) = $-0.0016 + 0.0020 \times \log$ temperature + 1</td>
<td>0.043</td>
<td>0.181</td>
<td>24</td>
</tr>
</tbody>
</table>

($P = 0.022$) on 24 June. There were no significant differences among taxa in N : P excretion ratio on other dates ($P > 0.05$).

For all three taxa, mass-specific N excretion rate increased significantly with temperature (Table 2). For P excretion, the regression for Chaoborus was not significant, while those for chironomids and tubificids were significant. The percentage of variance in excretion rates explained by temperatures was relatively low ($\leq 33\%$ in all regressions; Table 2).

**Nutrient fluxes**

Nutrient fluxes via benthic invertebrate excretion varied considerably among the three sites (Fig. 4). Fluxes of both N and P were highest at the Inflow site, intermediate at the Middle site and lowest at the Dam site. Furthermore, the relative contribution of the three taxa varied among the sites. At the Inflow and Middle sites, tubificid excretion dominated N and P fluxes, while at the Dam site excretion by
Chaoborus constituted the greatest fraction (Fig. 4). Excretion by chironomids was not dominant at any site, and this taxon made a substantial contribution to total flux only at the Inflow site. Flux rates were highest at the Inflow site and lowest at the Dam site because of two factors. First, biomass of tubificids and chironomids were much greater at the Inflow site than at the Dam site (Fig. 2). Secondly, temperature at the sediment–water interface was usually several degrees higher at the Inflow site than at the Dam site. The Middle site was intermediate in terms of both total invertebrate densities and temperature and, hence, flux rates were intermediate at this site. As mass-specific excretion rates were not statistically different among species, differences in flux rates among sites are most likely to be the result of variation in total invertebrate abundance, and not because of species composition.

The N : P excretion ratio of the entire assemblage varied seasonally and among sites (Fig. 5). This ratio increased by late June at the Inflow and Middle sites, and then declined from September until early November. The N : P excretion ratio was relatively constant at the Dam site and tended to be lower than that at the other sites. The lake-wide N : P excretion ratio was similar to that at the Inflow and Middle sites, because fluxes from these two sites dominate lake-wide flux (Fig. 5).

Discussion

Benthic invertebrate abundance and biomass

The three taxa each differed in terms of spatial distribution, probably the result of an interaction of physicochemical and biotic factors. It is well known that Chaoborus larvae sometimes inhabit bottom sediments during daylight, escaping predation by visually feeding fish, and migrate into the water column at night. In this study, Chaoborus biomass was lowest at the Inflow site, where Chaoborus has little protection from visually feeding fish. Apparently, Chaoborus individuals avoid settling in the sediments of the Inflow site, or experience high predation rates after settling there. Chaoborus was the only abundant taxon at the anoxic Dam site.
Chironomids and tubificids were rare at the Dam site, which was anoxic from late May until September. Chironomids are able to endure short periods of low oxygen supply because of the possession of a respiratory pigment (Armitage, Cranston & Pinder, 1995). However, our data suggest that they do not tolerate long periods of anoxia, such as those experienced at the Dam site. Chironomids were also scarce at the Middle site, which is only infrequently anoxic. The spatial distribution of chironomids and tubificids in Acton Lake suggests that predation by visually feeding fish does not restrict these taxa to deeper sites, but it is difficult to ascertain the role of fish predation in determining distributions. By far the most abundant fish is the gizzard shad, *Dorosoma cepedianum* (LeSueur), which consumes sediment and...

**Fig. 4** Fluxes of nitrogen (as NH$_4$-N) and phosphorus (as soluble reactive P) through benthic invertebrates at three sites in Acton Lake in 1997. In each panel, the top line represents the total flux through the assemblage (three taxa combined), while the shadings represent fluxes contributed by each taxon.

**Fig. 5** N : P ratio excreted by the benthic invertebrate assemblage (three taxa combined) at three sites, and lake-wide, in Acton Lake in 1997.
zooplankton (Schaus et al., 1997, 2002). Benthic invertebrates are virtually absent from gizzard shad guts (based on quantification of gut contents of hundreds of individuals; Schaus, Vanni & Wissing, 2002; K.A. Sigler and M.J. Vanni, unpublished data). Predation rates by other fish (e.g. centrarchids) are probably highest at the Inflow site, as light intensity at the sediment–water interfaces is much higher there than the other sites. Predation rates are also undoubtedly lowest at the Dam site, because anoxic waters exclude fish.

Daniel (1972, 1984) quantified benthic invertebrate densities in Acton Lake from 1964 (7 years after the lake was formed) until 1976, at our three sites as well as two others. We compiled data from June until November 1964, 1965, 1969 and 1970 from Daniel (1972) so that we could compare them to our results. Post-1970 data presented in Daniel (1984) were not used because sample collection dates did not correspond to our sampling period (June–November). In general, we found distribution patterns similar to those reported by Daniel (1972). Chaoborus, chironomids and tubificids constituted the three most abundant benthic invertebrate taxa in the lake between 1964 and 1970, as we also observed. Chaoborus was the only abundant taxon at the Dam site, and chironomids and tubificids dominated at the Middle and Inflow sites during this period as well as during our study (Table 3). Daniel (1972, 1984) also reported increasing abundance of tubificids over the 12 years of his studies. Based on our results, this trend has continued in more recent years (Table 3).

The Acton Lake benthic assemblage is typical of that of highly eutrophic lakes, which are often dominated by chironomids and tubificids (Jónasson, 1984; Popp & Hoagland, 1995; Svensson et al., 1999). In addition, the increase in tubificids over time agrees with the study of Popp & Hoagland (1999), who found that tubificids increased in Pawnee Reservoir as it became more productive with increasing age.

### Excretion rates

Interspecific differences in mass-specific nitrogen and phosphorus excretion rates were slight. Tubificids tended to show higher mass-specific excretion rates than chironomids (Fig. 3), but differences were generally not significant. It is not too surprising that temperature explained relatively little variance in excretion rates. For example, Gardner et al. (1981) found no clear effect of temperature on the release of phosphorus by tubificids or chironomids. While temperature must exert a metabolic control on basal excretion rate, excretion in nature may be affected more by food ingestion rate, as this has a great influence on the excretion of most animals (Johannes, 1964; Lehman, 1980; Gardner & Scavia, 1981; Gardner et al., 1981, 1983; Mather et al., 1995). Overall, our results suggest that factors such as temperature and biomass are more important in determining N and P fluxes than is species composition in this assemblage. In other words, in terms of nutrient excretion, these taxa appear to be somewhat functionally redundant (sensu Lawton & Brown, 1993).

<table>
<thead>
<tr>
<th>Site</th>
<th>Population density (individuals m⁻²)</th>
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<th></th>
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<tbody>
<tr>
<td></td>
<td>Chaoborus</td>
<td>Chironomidae</td>
<td>Tubificidae</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>Inflow</td>
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<tr>
<td>Daniel (1972)</td>
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<tr>
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<td>177.8</td>
<td>17.0</td>
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The excretion rates we report here are higher than many others reported for benthic invertebrates (Table 4). However, this can potentially be explained by two factors: incubation time and temperature. In most previous studies, animals were incubated for several hours or more after being separated from food (Table 4), while in our experiments incubations were only for 1 h (for experiments conducted at temperatures \( \geq 15^\circ C \)) or 4 h (<\( 15^\circ C \)). Several studies have shown that excretion rate declines shortly after animals are separated from their food source (Johannes, 1964; Lehman, 1980; Gardner & Scavia, 1981; Gardner et al., 1981; Mather et al., 1995; Arnott & Vanni, 1996), although this may not always be the case (e.g., Gardner et al., 1983). Comparison with other studies using similar incubation times shows that the excretion rates we report here are reasonable. For example, Gardner et al. (1981) report a P excretion rate of 3.5 nmol P mg\(^{-1}\) h\(^{-1}\) for tubificids using an incubation time of 0.5 h at 20\(^\circ\)C, very similar to our mean rate of 3.4 nmol mg\(^{-1}\) h\(^{-1}\) for tubificids with a 1-h incubation time at 20\(^\circ\)C (Table 4). Similarly, Gardner et al. (1983) report an N excretion rate of 14.7 nmol N mg\(^{-1}\) h\(^{-1}\) for chironomids at 22\(^\circ\)C (incubation time 2 h), compared with our finding of 24.4 nmol N mg\(^{-1}\) h\(^{-1}\) for chironomids at 25\(^\circ\)C (incubation time 1 h; Table 4). Our rates may be somewhat higher than Gardner et al.’s because our temperature was higher and incubation time

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Temperature (°C)</th>
<th>Incubation time (h)</th>
<th>Nitrogen</th>
<th>Phosphorus</th>
<th>Source</th>
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<td>–</td>
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<td>4.20</td>
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<td>0.98</td>
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<td>4.75</td>
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<td>25</td>
<td>1</td>
<td>24.40</td>
<td>1.46</td>
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</table>

*Indicates that rate was not measured.
shorter. In addition, recent studies using short incubation times to quantify excretion by amphipods report rates similar to, or greater than, ours. Wilhelm et al. (1999) report P excretion rates of 1.5 (adults) and 6.7 (adults and juveniles together) nmol P mg⁻¹ h⁻¹ for *Gammarus lacustris* (Sars) at 10°C in an alpine lake. The latter rate exceeds the rates reported here for all three taxa in our November experiment, when temperature was 9°C (Table 4). Also, Tuominen et al. (1999) report N excretion rates of 5.9 nmol N mg⁻¹ h⁻¹ for the marine amphipod *Montoporeia affinis* (Lindström) at 5°C; this also exceeds the excretion rates for all three of our taxa in the November experiment (Table 4).

To evaluate quantitatively the effect of temperature and incubation times on N and P excretion rates, we analysed the data from Table 4 using regression (Fig. 6). We assigned each of the three taxa (*Chaoborus*, chironomids and tubificids) a taxon code (1–3) and used multiple regression on log-transformed data to explore how taxon identity, temperature and incubation time are related to excretion rates. We excluded from this analysis experiments for which incubation times were not precisely defined (i.e. ‘>24 h’ in Table 4). Nitrogen excretion rate increased with temperature ($P = 0.041$), decreased with incubation time ($P = 0.015$) and was affected by taxon identity ($P = 0.053$; Fig. 6). None of the possible two- or three-way interactions were significant (for all, $P > 0.10$). The model with these three independent variables (and no interaction terms) explained 46% (adjusted $R^2$) of the variance in N excretion rate. For P excretion rate, the temperature effect was not significant in the multiple regression model ($P = 0.87$), so temperature was dropped from the model. A reduced model with the two remaining independent variables showed a significant effect of incubation time ($P = 0.008$) and taxon identity ($P = 0.040$); the interaction of these two factors was not significant ($P = 0.37$). The model with these two independent variables (and no interaction term) explained 33% of the variance in P excretion rate. These analyses show that incubation time can have a large effect on N and P excretion rates, and must be carefully considered in studies in which the goal is to quantify rates in natural ecosystems.

Several studies have shown that benthic invertebrates can affect nutrient flux from sediments via bioturbation as well as nutrient excretion (Gallepp,
In some cases it appears that excretion accounts for the majority of nutrient release mediated by benthic invertebrates, while in other cases it appears that other mechanisms, including bioturbation, are more important. Bioturbation effects may actually counteract excretion flux. For example, bioturbation can increase the oxygen content of near-surface pore-waters, leading to an increased rate of nitrification of excreted ammonium (Tuominen et al., 1999) or precipitation of phosphorus. Physical actions of the invertebrates themselves may mediate these processes. Chironomid excretion products are more likely to be released into the overlying water because of the construction of burrows, as water flows freely through the burrows with respiratory movements (Fukuhara & Sakamoto, 1987). Conversely, oligochaete excretion products are trapped in the sediment mainly because of the lack of burrows, and as products are excreted through nephridia on each segment directly into the sediment (Fukuhara & Sakamoto, 1987). Tatrai (1986) found that release rates were two–four times higher when organisms were removed from the sediments than when they were left in sediments. However, Nalepa, Gardner & Malczyk (1983) demonstrated that absence of a substratum did not affect tubificid or chironomid phosphorus excretion rates, while Gardner et al. (1983) also found that nitrogen excretion rates were not affected. Clearly the net effects of benthic invertebrates on nutrient flux are a function of several potentially interacting processes. Further studies are needed, in which excretion rates (quantified over short incubation times) and bioturbation effects are explicitly compared in a variety of habitats.

**Table 5** Fluxes of ammonium and soluble reactive phosphorus into the Acton Lake water column via a variety of processes, using June–October data. For benthic invertebrate excretion (this study), direct release from sediments, and gizzard shad excretion, lake-wide flux rates were obtained by assuming that oxic and anoxic sediments each comprise half of the total sediment (lake surface) area (Evarts, 1997). For catchment inputs, total loading to the lake was divided by total lake surface area.

<table>
<thead>
<tr>
<th>Flux process</th>
<th>Ammonium flux (μmol N m⁻² day⁻¹)</th>
<th>Soluble reactive P flux (μmol P m⁻² day⁻¹)</th>
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<tbody>
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<td>Oxic sites</td>
<td>Anoxic sites</td>
</tr>
<tr>
<td>Benthic invertebrate excretion (1997)*</td>
<td>1396</td>
<td>145</td>
</tr>
<tr>
<td>Direct release from sediments (1996)†</td>
<td>938</td>
<td>1775</td>
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<tr>
<td>Gizzard Shad excretion (1994)‡</td>
<td>4740</td>
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</tr>
<tr>
<td>Catchment (Inflow streams)§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1997</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1995 (wet year)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1994 (dry year)</td>
<td></td>
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</tr>
</tbody>
</table>

*This study.
†Evarts (1997).
‡Schaus et al. (1997).
§Vanni et al. (2001).
much more N and P than did the benthos in 1997 (Table 5). In a dry year (1994), however, the catchment provided much less N and P than did the benthos in 1997. This suggests that the relative importance of these two factors will vary greatly depending on precipitation. In addition, because of the predominance of agricultural land, inputs of nitrate–N from the catchment exceed NH$_4$–N inputs by 50–100-fold, in both wet and dry years (Vanni et al., 2001). Thus, excretion of N by benthic invertebrates is not likely to exceed catchment inputs of dissolved inorganic N, even in dry years.

Comparison of excretion by benthic invertebrates with other internal fluxes shows that spatial variation is important. Release of both N and P from anoxic sediments (as determined by incubation of intact sediment cores; Evarts, 1997) is much greater than that excreted by benthic invertebrates in anoxic waters, but the pattern is reversed in oxic sites (Table 5). Thus, at the lake-wide scale, the relative importance of these two factors will depend on the relative area of anoxic and oxic sediments. In Acton Lake, where anoxic and oxic sediments are about equal in area, it appears that release of nutrients from sediments exceeds that from benthic invertebrates (Table 5). However it should be noted that ‘direct release from sediments’ in this case probably includes some excretion by benthic invertebrates because intact cores were incubated (Evarts, 1997), and at least some cores probably contained invertebrates. On the other hand, the excretion rates we present here for benthos may be overestimates, as mentioned above, because animals were separated from sediments. This may explain why benthic invertebrate excretion rates exceed direct release from sediments in oxic sites (Table 5). Fukuhara & Sakamoto (1987) also found that P excretion by tubificids and chironomids could be more than in anoxic sediments. To account for this discrepancy they suggested that some of the P excreted by invertebrates in intact sediments remained in pore waters and did not diffuse to the water column.

Another important source of nutrients in Acton Lake is excretion by gizzard shad, *Dorosoma cepedianum*. This fish (family Clupeidae) consumes mostly sediment detritus and usually comprises >80% of fish biomass in Acton Lake. Schaus et al. (1997) found that gizzard shad excrete considerable quantities of N and P. Indeed, based on 1994 data (from Schaus et al., 1997), it appears that flux through gizzard shad greatly exceeds flux through benthic invertebrates for both N and P (Table 4). However, it should be noted that gizzard shad biomass was very high in 1994 (>400 kg ha$^{-1}$). Over the period of 1994 until 1999, gizzard shad biomass has averaged ~200 kg ha$^{-1}$; at this mean density, excretion by gizzard shad would still exceed that from benthos (based on the 1997 benthos data presented here), but the difference between the two sources is greatly reduced (Table 5).

Benthic invertebrates excreted N and P at a ratio of 15–20 in oxic sites during summer months (Fig. 5). This is similar to the ratio at which gizzard shad excrete nutrients (Schaus et al., 1997) but much lower than the ratio at which the catchment delivers dissolved inorganic nutrients (i.e. including NO$_3$ as well as NH$_4$; Vanni et al., 2001). Excretion of nutrients at such a relatively low ratio could favour cyanobacteria, which are the dominant phytoplankton group in Acton Lake.

In summary, our results support the hypothesis that nutrient flux through benthic invertebrates can be an important source of nutrients to the water column. However, the relative importance of this flux is likely to vary greatly spatially and temporally. Future studies need to quantify this variation and explain its causes.

Acknowledgments

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References


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