"TOP–DOWN" TROPHIC INTERACTIONS IN LAKES: EFFECTS OF FISH ON NUTRIENT DYNAMICS

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Abstract. We conducted enclosure experiments over two summers in Tuesday Lake, Michigan, to assess how a gradient of zooplanktivorous fish biomass affected the dynamics of nutrients (nitrogen, N, and phosphorus, P), and their partitioning among ecosystem compartments. In both years, fish (the cyprinid Phoxinus eos) reduced the abundance of large zooplankton species and increased the biomass of phytoplankton as predicted by the top–down control hypothesis.

Fish had strong effects on the dynamics and fluxes of N and P. Total P concentrations in the water column declined over time in all enclosures, but fish slowed the rate of decline. Thus total water column P increased with increasing fish biomass. Total N increased less strongly with increasing fish biomass, and thus the total N:P ratio decreased with increasing fish biomass. The concentrations of particulate carbon, nitrogen, and phosphorus in the water column also increased with increased fish biomass. Particulate N:P ratio decreased with increased fish biomass, but effects were weaker compared to effects on total N:P ratios. Nutrient ratios of the zooplankton fraction (particles >63 μm) showed a response that was transient but consistent with observed trends in zooplankton species composition. In particular, when the large cladocerans Daphnia and Holopedium increased upon exclusion of fish, C:P and N:P ratios of the zooplankton fraction showed distinct declines, corresponding to the relatively high body P contents of these taxa.

Phosphorus budgets revealed that fish were a net source of P to the water column, because they lost mass during the experiments, even at densities below those in the lake. However, loss of P from fish could not account for the higher total P concentration observed in enclosures with fish compared to fishless enclosures. The absolute amount of P sinking from the water column increased with increasing fish biomass but decreased when expressed as percentage of total P sinking, again suggesting that the presence of fish increases the relative retention of P the water column. The rate of decline in water column total P in the presence of fish was accurately predicted by sedimentation of P from the water column and other fluxes. Our results support the hypothesis that fish can exert major influences on the dynamics, distribution, and ratios of limiting nutrients.

Key words: fish; food webs; lakes; nitrogen; nutrient budget; nutrient recycling; phosphorus; phytoplankton; sedimentation; stoichiometry; top–down effects; trophic cascade; zooplankton.

INTRODUCTION

Individual species can have strong effects on the physical and chemical characteristics of ecosystems (Jones et al. 1994, Jones and Lawton 1995). In particular, certain plant and animal species have effects on resource availability that are disproportionately large in relation to their abundance or biomass in a food web (e.g., Huntly and Inouye 1988, Naiman et al. 1988; Whicker and Detling 1988, Sterner 1990, Wedin and Tilman 1990, Hobbie 1992, Pastor et al. 1993, Jones et al. 1994, Wedin 1994).

It is increasingly clear that food web structure and the particular identity of animal species may have substantial effects on the dynamics of nutrients in lakes (e.g., Carpenter et al. 1992, Caraco 1993, Sterner 1995, Vanni 1996). For example, an abundance of zooplanktivorous fish may result in a higher concentration of water column total phosphorus (P) than in the absence of fish (Andersson et al. 1978, Shapiro and Wright 1984, Mazumder et al. 1988, Drenner et al. 1990, Reinertsen et al. 1990, Lazzaro et al. 1992). Fewer studies have quantified effects of fish on nitrogen (N) dynamics, but it appears that fish have lesser effects on water column total N than on P (Drenner et al. 1990, Lazzaro et al. 1992).

The effects of fish on nutrient dynamics may potentially involve many feedbacks between “community” and “ecosystem” processes. For example, the positive effects of zooplanktivorous fish on water column P, which is usually the limiting nutrient, may derive simply from the “top–down” effect of these fish (Carpenter and Kitchell 1988, 1993a). When zooplanktivorous
fish are abundant, they greatly reduce the abundance of large zooplankton, and presumably total herbivory rate on phytoplankton, which can lead to increased phytoplankton abundance. Because much P in the open waters of lakes is tied up in phytoplankton biomass, water column P concentration may therefore increase when zooplanktivorous fish are abundant. When zooplanktivorous fish are rare and herbivory rate high, phytoplankton biomass may be low, leading to low P concentration in the water column. Phosphorus, which would otherwise be present in phytoplankton cells, may enter other pools such as the littoral zone or sediments.

Food web structure may also affect how nutrients are recycled by animals in lakes. For example, considerable evidence exists that small zooplankton species, which predominate when piscivores are rare and zooplanktivorous fish are abundant, recycle nutrients at a higher rate per unit biomass than larger species (Wen and Peters 1994). Fish also excrete dissolved inorganic nutrients, and recent studies suggest that excretion by small zooplanktivorous fish can be an important source of P for phytoplankton (Vanni and Findlay 1994, McQueen et al. 1992, Schindler 1992, Schindler et al. 1993, 1996). Many “zooplanktivorous” fish also feed on littoral and benthic prey yet excrete nutrients into the pelagic (open water) zone; in so doing they may increase the rate at which nutrients are transported from littoral/benthic habitats to pelagic habitats (Lamarra 1975, Schindler et al. 1996, Vanni 1996).

Food web structure may also affect the ratio of nutrient supply to phytoplankton. Different zooplankton species seem to sequester and thus potentially recycle nutrients at different N:P ratios (Andersen and Hessen 1991, Sterner et al. 1992). For example, *Daphnia*, a large herbivorous species, which often dominates when zooplanktivorous fish are scarce, has a lower tissue N:P ratio compared to species such as copepods, which often dominate when zooplanktivores are abundant. These interspecific differences seem to be maintained regardless of the nutrient content of the herbivore’s food (Sterner and Hessen 1994). According to stoichiometric theory (Sterner 1990, Sterner et al. 1992), this suggests that to maintain nutrient homeostasis (i.e., low body N:P), *Daphnia* would excrete nutrients at a higher N:P ratio than copepods. Since zooplankton species composition is largely controlled by fish predation, fish can indirectly affect the rates and ratios at which N and P are recycled by zooplankton herbivores (Sterner et al. 1992).

Thus the effects of fish on nutrient dynamics are potentially strong and complex. While many studies have demonstrated some aspect of the effects of fish on nutrients, few comprehensive studies exist. In this study we address the following questions: (1) How do zooplanktivorous fish affect the concentrations and ratios of N and P in the water column? (2) How do zooplanktivorous fish affect the partitioning of these nutrients into dissolved and particulate pools? (3) Are effects of fish on particulate nutrients consistent with predictions of stoichiometric theory? (4) Can processes such as sedimentation, net gain or loss of nutrients by fish, and loss of nutrients to attached algae explain fish-induced fluxes of nutrients? We addressed these questions by varying the abundance of zooplanktivorous fish in large enclosures. We used a gradient of fish abundance (rather than simply presence/absence), because of potential nonlinearities in responses to fish abundance (e.g., Neill 1988, Kitchell et al. 1988) and because few studies have explored the effects of fish over a range of fish biomass (Threlkeld 1988, Drenner et al. 1990, Drenner and Smith 1991).

**Study Site**

This research was conducted in Tuesday Lake, which is small (1.2 ha surface area), relatively deep (maximum depth 16.5 m), and unproductive (annual mean total P in the water column was ≈300–450 µmol/L from 1984 to 1990; Carpenter et al. 1993b). Tuesday Lake lies within the University of Notre Dame Environmental Research Center (UNDERC) in the upper peninsula of Michigan, USA, and was chosen for several reasons. First, whole-lake manipulations of the Tuesday Lake fish community showed a clear “top-down” phytoplankton response (Carpenter et al. 1987, Carpenter and Kitchell 1988, 1993a) and the lake was selected in part because of this strong response. We reasoned that effects on nutrient dynamics may also be strong. A second reason for choosing this lake is that because piscivores do not occur naturally, all limnetic trophic levels could be included in standard-sized enclosures, which are too small for piscivores. Thirdly, unlike most lakes, fish biomass in Tuesday Lake has been quantified (Carpenter and Kitchell 1993a). We were thus able to conduct experiments with fish densities that reflect natural Tuesday Lake conditions. Finally, we felt that these enclosure experiments together with additional experiments (Vanni and Layne 1997) could shed light on the mechanisms accounting for the top-down effects of fish on phytoplankton communities.

After the original piscivore addition in 1985, piscivores were removed and the Tuesday Lake food web returned to premanipulation conditions (Hodgson et al. 1993, Soranno et al. 1993a, b, Carpenter et al. 1993a, b). Thus when we began our experiments in 1990 the fish assemblage was similar to premanipulation conditions and consisted of only nonpiscivores. A cyprinid, the northern red-belly dace (*Phoxinus eos*) was the most abundant fish species, and abundance of cyprinids (minnows) measured as catch-per-unit-effort (CPUE) was essentially identical to that of premanipulation conditions (Hodgson et al. 1993). Largemouth bass were added again to Tuesday Lake in July 1990 (Carpenter and Kitchell 1993b). However, they were added as young-of-year in 1990 and at a very low population biomass (only 2 kg added to the lake; Carpenter and Kitchell [1993b]). Since bass were scarce and small
would probably allow of zooplanktivorous fish over a long time period. This response is usually most evident when the top-down effects of fish on phytoplankton and nutrient fractions, as well as plankton abundances. A companion paper (Vanni and Layne 1997) quantified the roles of herbivory and nutrient recycling by the food web in causing phytoplankton community response.

Enclosures were made of cylindrical polyethylene tubes closed off from the lake at the bottom, open to the atmosphere at the top, and suspended from the surface of the lake. Enclosures were 1 m in diameter and ~3 m deep, yielding a total volume of ~2350 L. Enclosures were filled by pumping water through a 63-µm mesh plankton net to remove zooplankton. (Prior experience had shown that pumping zooplankton into enclosures causes significant mortality of some species). Tuesday Lake zooplankton were then added from vertical tows taken from the lake. Each enclosure received five vertical hauls from 6 m with a 30 cm diameter 63-µm mesh plankton net.

The goal of these experiments was to characterize the top-down effects of fish on phytoplankton and nutrients. This response is usually most evident when the abundance of zooplanktivorous fish is varied in a community containing large-bodied grazers, especially *Daphnia*. However, at the time of our experiment the Tuesday Lake zooplankton assemblage contained only small species (Soranno et al. 1993a). Although exclusion of zooplanktivorous fish over a long time period would probably allow *Daphnia* to colonize and become abundant (Soranno et al. 1993a), our enclosure experiments were relatively short in duration. Therefore, we began experiments with a “regional” zooplankton species pool, consisting of large and small species. We added zooplankton from nearby lakes in which large species, including *Daphnia*, were abundant; we added zooplankton from Peter Lake in 1990, and from West Long Lake in 1991. In both years, we did so by taking vertical hauls from the donor lake with a plankton net (30 cm diameter, 153-µm mesh) and adding the zooplankton to all Tuesday Lake enclosures. In 1990 we added zooplankton from five hauls taken from 6 m depth in Peter Lake to each enclosure and in 1992 we added three hauls taken from 4 m depth in West Long Lake to each enclosure.

After zooplankton were added to enclosures, zooplanktivorous fish (northern red-belly dace, *Phoxinus eos*) were added from Tuesday Lake. In 1990, we established four treatments: No Fish, Low Fish, Medium Fish, and High Fish. Each treatment consisted of three replicate enclosures. In 1991 we used just three treatments, with four enclosures per treatment (Table 1). Stocking rates of fish ranged from 32.5 to 113.9 kg/ha (Table 1). Minnow biomass in Tuesday Lake (most of which was *P. eos*) was estimated to be ~50 kg/ha when we began our experiments (Carpenter et al. 1987, Hodgson et al. 1993). Thus fish densities in our enclosures spanned a range below and above that found in Tuesday Lake. Fish were captured from Tuesday Lake with minnow traps, placed in buckets, and anesthetized with MS-222. Fish were then measured (in both years) and weighed (in 1991 only), allowed to recover in buckets without anesthetic, and added to enclosures. Fish were placed in enclosures within 1 hour of being captured.

In 1990, enclosures were filled with water and phytoplankton on 29 June, Tuesday Lake zooplankton added on 30 June, and large zooplankton and fish added on 4 July. The experiment was terminated on 13 August. In 1991, enclosures were filled and all zooplankton added on 7 June, and fish were added on 12 June, and the experiment was terminated on 11 July. In both years, enclosures were sampled once before fish were added.

### Sampling methods

In 1990 and 1991, we quantified the concentrations of N and P in the water column, including total and

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<th>Recovered</th>
<th>Biomass of fish stocked (kg wet mass/ha)</th>
<th>Number of fish per bag Stocked</th>
<th>Recovered</th>
<th>Biomass of fish stocked (kg wet mass/ha)</th>
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<td>97.7</td>
</tr>
</tbody>
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† Ellipsis indicates that treatment was not employed in 1991.
particulate fractions. We also quantified water column particulate carbon concentrations (but not total carbon) in both years, and constructed a P “budget” for the enclosures. To do so, we quantified the amounts of P sequestered in organisms growing on the inside walls of enclosures (hereafter, “wall growth”), P lost from the water column through sedimentation, and net loss or accumulation of P through fish mass loss or gain. We attempted to construct such a budget to shed light on how fish affect the fate of nutrients and whether fish mediate the “loss” of P from the water column. We also quantified zooplankton community composition and nutrient content, to assess whether shifts in particulate nutrients corresponded with shifts in zooplankton communities as predicted by stoichiometry theory. Phytoplankton biomass and species composition data were also quantified; species composition data are presented in the companion ms (Vanni and Layne 1997).

Water column nutrients.—In both years, we quantified “Total N and P” in the water column. This represents all N and P suspended in the water column, excluding fish. In 1990, water for total N and P analyses was obtained by lowering a weighted piece of plastic tubing (hereafter “integrated tube sampler”) to near bottom of the enclosures (=2.75 m), thereby trapping an integrated water column sample. Samples were frozen in Nalgene bottles without filtration or screening to remove zooplankton. Thus samples include both particulate and dissolved fractions. However, we believe that this commonly used method may underestimate the contribution of large zooplankton (e.g., Daphnia, Chaoborus) to total N and P. These large species can make up a substantial contribution to total P in UNDERC lakes when zooplanktivorous fish are scarce (Schindler et al. 1993) and many individuals can probably avoid the tube sampler. In 1991 we therefore screened water from the integrated tube sampler through a 63-μm mesh. Water and organisms passing through the mesh were frozen; these screened samples thus included dissolved N and P as well as N and P particles passing through a 63-μm mesh. The <63-μm particulate fraction is considered to be seston, which includes phytoplankton, bacteria, and detritus. In 1991, zooplankton were sampled from enclosures with a 12-L Schindler–Patalas trap on all sampling dates. From each enclosure, we took five casts of the trap (at depths of 0.5, 1, 1.5, 2, and 2.5 m). These samples were pooled and then divided with a plankton splitter into three aliquots. One aliquot was preserved in sucrose-foral for zooplankton species quantification, while the other two were filtered onto two Gelman A/E glass fiber filters, one for particulate C and N analysis and one for particulate P analysis. In 1991 total nutrient concentration was obtained by summing the dissolved plus seston fraction (from tube sampler) and the zooplankton fraction (from Schindler trap).

We analyzed for total P (TP) using the ammonium molybdate method following persulfate digestion (Wetzel and Likens 1991). In 1990, we quantified total N (TN) using a microKjeldahl technique used previously in Tuesday Lake (Elser et al. 1988). However, it became apparent that this procedure underestimated TN. Therefore in 1991 we switched to second-derivative spectrophotometry (Crompton et al. 1992). Although the digestion procedure we used in 1990 was incomplete, it apparently was consistent, and we were able to calculate actual TN by intercalibrating the two methods. To do so, on one date early in the 1991 experiment, we analyzed samples from all 12 enclosures and Tuesday Lake (13 samples total) for TN using our 1990 microKjeldahl technique and the second-derivative spectrophotometry technique. We also used a third technique employing a block digestor followed by analysis with a Lachat auto-analyzer (samples run by S. Knight, Trout Lake Station, University of Wisconsin). Mean (± 1 SD) TN “concentration” was 10.4 ± 0.46 μmol/L as determined with our 1990 microKjeldahl technique, and 28.2 ± 0.26 μmol/L with the second-derivative spectrophotometry technique. TN concentrations as determined with the block digestion/autoanalyzer method (mean = 28.5 ± 0.26 μmol/L) were essentially identical to those obtained with second-derivative spectrophotometry. The second-derivative spectrophotometry technique thus yielded on average 2.71 × (SD = 0.385) more TN than our 1990 microKjeldahl technique, and we therefore multiplied all 1990 TN values by 2.71 to correct for incomplete digestion. Note that this is not meant as a general condemnation of the microKjeldahl technique; rather the inefficiency resulted from a digestion too brief in duration (S. R. Carpenter, personal communication).

We also quantified concentrations of particulate N, P, and C. In 1990, water was collected from each enclosure with the integrated tube sampler to obtain a sample for all particulate nutrients. Each sample was then split with a plankton splitter, and each aliquot filtered onto a Gelman A/E glass fiber filter previously ashed at 475°C for 8 h. After filtration, filters were placed overnight in a vacuum dessicator, then wrapped in aluminum foil, and frozen. One filter was then assayed for particulate P using the HCl digestion method of Stainton et al. (1977), while the other was assayed for C and N using a Perkin Elmer Series 2400 elemental analyzer. Our 1990 particulate nutrient concentrations represent seston (phytoplankton, bacteria, and detritus), as well as any zooplankton captured with the integrated tube sampler. In 1991, we separated the particulate fraction into seston and zooplankton components. Thus seston nutrients were quantified using samples collected with the integrated tube sampler, passed through the 63-μm mesh to exclude zooplankton, and collected onto Gelman A/E glass fiber filters. The glass fiber filters from zooplankton samples were used to estimate nutrients in the zooplankton fraction in 1991, using the same methods as that for seston nutrients. Thus, in both years, “total N and P” refer to dis-
solved fractions plus all particulates (except fish) suspended in the water column. In 1990, “particulate N and P” include both zooplankton and seston. In 1991, particulate nutrients were subdivided into zooplankton (>63 µm) and seston (<63 µm) fractions.

Fish survival, growth, and nutrient content.—We assessed the survival, growth, and nutrient content of fish added to enclosures to determine their net nutrient release or accumulation. Fish were recovered by placing minnow traps in enclosures at the end of each experiment. In 1990, all fish were recovered on one date (16 August). In 1991 we set traps for several days (11–15 July) but did not recover all fish (Table 1).

We present changes in the nutrient content of fish only in 1990, because in 1991 some fish did not survive. At the end of each experiment, each fish was measured to the nearest centimeter and weighed to the nearest 0.01 g (wet mass). Fish were then sacrificed, dried overnight at 60°C, and reweighed to obtain dry mass, then frozen. Each fish was then pulverized into a fine powder with a mortar and pestle. Subsamples of pulverized fish were analyzed for C, N, and P concentration (per unit mass), using the methods for particulates described above. Nutrient concentration per unit dry mass was multiplied by fish dry mass per enclosure to estimate the total amount of nutrients present in fish at the end of the experiment.

Estimation of nutrient concentrations in fish at the beginning of the experiment was necessarily made through indirect methods, since nutrient content cannot be obtained without sacrificing fish. We measured all fish to the nearest centimeter before they were added to enclosures. We estimated initial wet masses of these fish using these length measurements and a length–wet mass regression developed for northern red-belly dace caught from Tuesday Lake with minnow traps during the enclosure experiment (1 August 1990, n = 75 fish). We also measured dry mass and nutrient contents of 10 of these fish to obtain wet:dry mass ratios and body nutrient contents. To obtain the amounts of nutrients initially present as fish in each enclosure, for each fish we first converted measured length to dry mass and then to C, N, and P per individual using the length–mass regression, dry mass:wet mass relationship, and nutrient content per dry mass from the fish caught from Tuesday Lake.

Nutrient budgets.—We used the estimated change in the fish nutrient pool as one contributing factor to our nutrient budgets. In addition, we quantified the amounts of nutrients sequestered in wall growth and lost through sedimentation. Because we measured N and P in the water column, sedimenting material, wall growth, and fish in both years, we could have constructed budgets for N and P in both years. However, we present a budget only for P in 1990. A budget was not constructed for N in either year because of potential atmosphere–water interactions, nor for P in 1991 because we were uncertain of the fate of nutrients in fish that died in enclosures.

To quantify wall growth nutrient content, we placed strips of enclosure plastic along the insides of the enclosures. Four strips were placed in each enclosure 90° apart from each other to account for variations in solar radiation in different parts of the enclosures. Strips were hung from the top lip of the enclosures and extended all the way to the bottom, to account for vertical variation in algal growth due to decreasing solar radiation with depth. Strips were weighted with a light weight for stability, removed at the end of the experiment by gently raising them to the surface, and placed in a 4-L plastic jar filled with filtered (Gelman A/E) lake water. Strips were returned to the laboratory, where they were vigorously shaken and scrubbed with a toothbrush to remove periphyton and other organisms. Detached organisms were then filtered onto Gelman A/E filters and processed as other particulate samples.

Nutrient loss by sedimentation from the water column was estimated by placing sediment traps near the bottom of enclosures. These traps were similar to those used previously to quantify pigment sedimentation rates in Tuesday Lake (Carpenter et al. 1986). Each trap consisted of four cylinders, 30 cm deep × 5 cm diameter, and were suspended from a nylon rope hung from a cross-rope at the top of enclosures. In 1990 sediment traps were placed in enclosures on 29 June and sampled twice, on 25 July and 15 August (end of experiment). Sediment trap contents were poured into Nalgene containers, filtered onto Gelman A/E filters, and processed as other particulate samples. Sediment traps were returned to the enclosures within 30 min of their removal on 25 July. A net sedimentation rate was estimated for each enclosure over the two time periods (29 June–25 July and 25 July–15 August) by subtracting the particulate P in traps at the beginning of each time period (assumed to be equal to water column) from the particulate P in traps at the end of each time interval. For each enclosure, sedimentation rates from the two time periods were added to yield a sedimentation rate for the entire experiment.

To determine if our budgets “balanced” we compared observed changes in total P in the water column over the course of the experiment with estimates of fluxes. If budgets balance, measured “outputs” from the water column (wall growth, sedimentation, net accumulation of P by fish) minus “inputs” (net loss from fish to water column) should equal the observed change in water column P.

Zooplankton and phytoplankton.—Zooplankton were sampled twice per week from enclosures. In 1990, zooplankton were sampled with a 12 cm diameter Wisconsin style plankton net with a 63-µm mesh. At the end of the experiment, efficiency of this sampling method was calculated by sampling, on the same date, with this net and with a 12-L Schindler–Patalas trap.
(63-µm mesh), which was assumed to be 100% efficient. In 1991, zooplankton were sampled from the enclosures with a Schindler–Patalas trap on all sampling dates.

For all preserved zooplankton samples, abundances (individuals per liter) of large species (crustaceans excluding copepod nauplii) were obtained by counting all individuals in the sample using a dissecting microscope. Abundances of small species (rotifers and copepod nauplii) were obtained by counting two subsamples using a compound microscope. We also estimated zooplankton biomass. To estimate biomass of size-structured crustacean populations we measured up to 30 individuals of each species per sample, on five dates per experiment. Lengths were measured with a compound microscope and converted to dry mass (micrograms per individual) using length–mass regressions of Dumont et al. (1975), Bottrell et al. (1976), and Lawrence et al. (1988). For rotifers we measured individuals along several axes (depending on species) and calculated body volume using geometric shapes provided by Ruttner-Kolisko (1977). Measurements of 20–30 individuals were made on randomly selected samples, and a mean volume obtained for each species. Within a rotifer species, volumes were assumed to be constant among dates and treatments. Dry mass for each rotifer species was obtained by assuming a specific gravity of 1 and a dry mass:wet mass ratio of 0.05 (Schindler and Layne 1997).

Phytoplankton were sampled twice per week from enclosures with the integrated tube sampler. In 1991, phytoplankton from Tuesday Lake were also sampled by lowering the tube sampler to a depth of 2.75 m. Lake phytoplankton were not sampled in 1990. Phytoplankton samples were preserved in Lugol’s solution (Wetzel and Likens 1991) and cells counted using an inverted microscope. Cells of each taxon were measured and converted to biovolume using formulas corresponding to appropriate geometric shapes. Volume was then was multiplied by cells per liter for each taxon to obtain population biovolume. Only total phytoplankton biovolume is presented here; results on phytoplankton species composition are presented in the companion ms (Vanni and Layne 1997).

**Temperature.**—Water temperature was measured with a YSI Model 58 temperature/oxygen meter at 0.5–1.0 m intervals in enclosures on all sampling dates. As it became apparent that temperature at a given depth did not vary among enclosures, we usually measured temperature in only a subset of enclosures rather than all of them.

**Statistical analyses and interpretation**

**Statistical analyses.**—Treatment differences in the abundances of zooplankton species and nutrient concentrations in enclosures were assessed using one-way ANOVA followed by Tukey’s test to separate specific treatments. For each analysis, a time-weighted mean for each enclosure was obtained, using all dates after fish were introduced and this mean was used as an observation in a one-way ANOVA (i.e., n = 3 in 1990 and 4 in 1991). Inspection of the data revealed that plankton and nutrient abundances varied over time, as did treatment differences. Therefore, additional ANOVAs were conducted using data at the end of the experiments (average of the final two dates). For all ANOVAs, data were log transformed before analysis to stabilize variances. Because of the necessarily small number of replicates some latitude is warranted when judging statistical significance, and we consider any P value <0.10 to indicate statistical difference (although we present all P values).

**Results**

**Temperature**

Enclosures were thermally stratified throughout both experiments, although they were not deep enough to contain a distinct hypolimnion (Fig. 1). Temperature profiles were similar in 1990 and 1991. The vertical gradient in temperature (and hence water density) suggests that sediment traps were effective in capturing particles without resuspension.
Zooplankton and phytoplankton dynamics

In both years biomass of the two large species of herbivorous zooplankton, *Daphnia* and *Holopedium*, were more abundant in the No Fish treatment than in treatments with fish in both years (Table 2). No significant differences were detected in the biomass of *Daphnia* or *Holopedium* among treatments containing fish (Table 2). Copepods, characterized almost exclusively by cyclopoids, were significantly reduced in abundance by fish in 1990 (Table 2). Copepod biomass was higher in 1991 than 1990, mostly because nauplii were more abundant in 1991 (Vanni and Layne 1997). Small taxa such as rotifers and the cladoceran *Bosmina longirostris* were least abundant in the No Fish treatments, possibly the result of competition for food with large herbivores and/or predation by the dipteran *Chaoborus*, which was most abundant in the No Fish treatment (Table 2; Vonder Brink and Vanni 1993). Total zooplankton biomass (excluding *Chaoborus*) was significantly lower in the High Fish treatment than in the other treatments only in 1990 (Table 2).

The two large herbivores *Daphnia* and *Holopedium* declined in the No Fish treatment in late July 1990 such that treatment differences for these species were not apparent by the end of this experiment (Fig. 2; Table 2). *Daphnia* and *Holopedium* were more abundant in the No Fish treatment than the other treatments throughout the entire experiment in 1991 (Fig. 2; Table 2). For all other taxa, treatment differences were similar whether all dates or the final two dates are considered (Table 2; Fig. 2). In 1991, zooplankton biomass was much higher in Tuesday Lake than in enclosures (note different scales for lake and enclosure graphs in Fig. 2).

Phytoplankton biovolume responded to a typical “top-down” fashion: algal biovolume increased with increasing fish biomass (Fig. 3). Considering all dates after fish introductions, phytoplankton biovolume was significantly higher in the High Fish treatment than in the No Fish treatment in both years (Table 2). Biomass in the Low Fish treatment was significantly higher than that in the No Fish treatment and lower than that in the Medium and High Fish treatments in 1990 (Table 2). Total phytoplankton biomass in Tuesday Lake during the 1991 experiment was usually less than that in enclosures; however, by the end of the experiment, phytoplankton biomass was intermediate that of the No Fish and Low Fish treatments (Fig. 3).

### Nutrient dynamics

**Water column nutrients.**—Fish had significant effects on the dynamics of nitrogen and phosphorus in the water column in both years (Figs. 4–7; Table 3). Fish significantly increased the concentration of TP in the water column in both years and of TN in 1991 (Fig. 4; Table 3). Fish increased TP relatively more than TN, and thus the TN:TP ratio decreased significantly with increasing fish biomass in both years (Fig. 4; Table 3). In 1991, general trends in TN, TP, and TN:TP were similar in the lake and enclosures (Fig. 4).

In 1990, the concentrations of particulate carbon, nitrogen and phosphorus were significantly higher in the presence of fish than in the absence of fish (Fig. 5; Table 3). Considering all dates after fish introductions in 1991, fish had no effect on seston C, N, or P. However, concentrations of seston C and seston P were higher in the presence of fish at the end of the experiment (Fig. 5; Table 3). In 1991, Tuesday Lake seston C, N, and P concentrations tended to be slightly lower than in the enclosures, and tended to be closest to the No Fish treatment (Fig. 5).

Particulate nutrient ratios tended to be less responsive than particulate nutrient concentrations to manipulations of fish biomass (Fig. 6; Table 3). Fish had no significant effect on particulate (1990) or seston (1991) C:N, C:P, or N:P ratios averaged over the entire experiments (Fig. 6; Table 3). However, fish had a sig-

### Zooplankton and phytoplankton dynamics

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<td>comparisons</td>
<td>comparisons</td>
<td>comparisons</td>
</tr>
<tr>
<td></td>
<td><em>P</em></td>
<td><em>P</em></td>
<td><em>P</em></td>
<td><em>P</em></td>
</tr>
<tr>
<td><em>Daphnia pulicaria</em></td>
<td>&lt;0.001</td>
<td>0.156</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>H M L N</td>
<td></td>
<td>H L N</td>
<td></td>
</tr>
<tr>
<td><em>Holopedium gibberum</em></td>
<td>&lt;0.001</td>
<td>--</td>
<td>0.011</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>L H M N</td>
<td></td>
<td>H L N</td>
<td></td>
</tr>
<tr>
<td><em>Bosmina longirostris</em></td>
<td>&lt;0.001</td>
<td>0.004</td>
<td>0.017</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>N H M L</td>
<td></td>
<td>N H L</td>
<td></td>
</tr>
<tr>
<td>Copepods</td>
<td>0.002</td>
<td>0.017</td>
<td>0.917</td>
<td>0.626</td>
</tr>
<tr>
<td></td>
<td>H M N L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rotifers</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.011</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td>N H M L</td>
<td>N H M L</td>
<td>N L H</td>
<td>N L H</td>
</tr>
<tr>
<td>Total zooplankton</td>
<td>0.001</td>
<td>0.002</td>
<td>0.955</td>
<td>0.443</td>
</tr>
<tr>
<td></td>
<td>H M L N</td>
<td>H N M L</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Chaoborus punctipennis</em></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.003</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>H M L N</td>
<td>H M L N</td>
<td>H L N</td>
<td>H L N</td>
</tr>
<tr>
<td>Total phytoplankton</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.010</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>N L M H</td>
<td>N L M H</td>
<td>N L H</td>
<td>N L H</td>
</tr>
</tbody>
</table>

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**Table 2.** Results of ANOVA on biomass of zooplankton and phytoplankton in the 1990 and 1991 experiments. Letters indicate treatment means as follows: N: No Fish, L: Low Fish, M: Medium Fish, and H: High Fish. Treatment means are ordered from lowest to highest; lines connecting letters indicate those treatment means were not significantly different (*P* > 0.10). Ellipsis indicates that *Holopedium* was too rare for analysis at that time.
Fig. 2. Zooplankton biomass in enclosures and Tuesday Lake during the 1990 and 1991 experiments. Note that the scale for the lake is different than that for enclosures.
Fig. 3. Total phytoplankton biovolume in enclosures and the lake.

Fig. 4. Total nitrogen, total phosphorus, and total N:P in the 1990 and 1991 experiments.
significant effect on particulate or seston C:P ratio at the end of each experiment, with the highest C:P ratio in No Fish treatments (Fig. 6; Table 3). In 1991, seston N:P ratio tended to be higher in the No Fish treatment than in the other treatments at the end of the experiment (Fig. 6; Table 3). Seston C:N ratio tended to be higher in Tuesday Lake than in enclosures in 1991, at least toward the end of the experiment (Fig. 6). Seston C:P in the lake was variable but slightly higher than in enclosures, while N:P ratios were similar between lake and enclosures (Fig. 6).

Overall, fish had no significant effects on the zooplankton fraction of C, N, or P concentrations or ratios of these elements (Fig. 7; Table 3). This corresponds to the lack of effect of fish on total zooplankton biomass in 1991 (Fig. 2; Table 3). The dynamics of the zooplankton nutrient pool paralleled those of total zooplankton biomass (compare Figs. 2 and 7). Trends in the zooplankton nutrient pools suggest that fish had transient effects on zooplankton nutrient contents and ratios, in the direction predicted by stoichiometry theory. In particular, during peak *Daphnia* abundance, zooplankton P concentration increased and zooplankton C:P and N:P ratios decreased (Figs. 2 and 7). Using only the time period of 17–24 June, which corresponds to the *Daphnia* peak, one-way ANOVA showed significant or marginally significant effects of fish on zooplankton P concentration (P = 0.062), C:P ratio (P = 0.027), and N:P ratio (P = 0.103). In each case the treatment effects were in the direction predicted by stoichiometry theory (higher P concentration and lower C:P and N:P ratios where *Daphnia* was abundant; Fig. 7). Fish had no significant effects on C or N concentration, or the C:N ratio, in the zooplankton fraction during this period (P = 0.591, 0.261, and 0.133, respectively). The concentrations of nutrients in the zooplankton fraction were much higher in Tuesday Lake than in enclosures, as was the case for zooplankton biomass (Figs. 2 and 7).

**Phosphorus budgets.**—Fish lost mass in all 1990 treatments, and this was accompanied by a net loss of nutrients from fish (Table 4). The C and N concentrations in fish tissue (percentage of dry mass) declined with increasing fish biomass (Table 4), possibly from catabolism of lipids and proteins. However, fish lost proportionately much less P relative to biomass or C and N (Table 4), and final P concentrations in fish tissue actually increased significantly with increasing fish biomass (Table 4).

Combining the loss of dry mass and final tissue nutrient content allows estimation of the total net loss of P from fish on an enclosure-wide scale (Table 4). Total net loss of P from fish to the water column was 5.9, 33.8, and 79.7 μmol P/L in the Low, Medium, and High Fish treatments over the course of the entire experiment (44 d). Note that this does not represent total flux of P through the fish, but rather the net balance of P (ingestion — egestion — excretion). Water column TP was significantly higher in the presence of fish than in their absence (Fig. 4; Table 3); thus it is useful to examine whether this effect can be explained by the loss of P from fish tissue to the water column. The difference in TP between the No Fish and the Low Fish treatments at the end of the experiment was 70.1 μmol P/L, while the amount of P lost from fish was only 5.9 μmol P/L. Thus, loss of P from fish accounts for only ~8% (5.9/70.1) of the observed difference in water column TP between these two treatments. Similarly, the loss of P from fish can

**Table 3.** Results of ANOVA on nutrient concentrations. “Particulate” nutrients include particles retained by a glass fiber filter and were measured in 1990. “Seston” nutrients include particles passing through a 63-μm mesh and retained by a glass fiber filter and were measured in 1991. See Methods for details. Letters indicate treatment means as follows: N: No Fish, L: Low Fish, M: Medium Fish, and H: High Fish. Treatment means are ordered from lowest to highest; lines connecting letters indicate those treatment means were not significantly different (P > 0.10). Ellipsis indicates that nutrient fraction was not quantified.

<table>
<thead>
<tr>
<th>Nutrient fraction</th>
<th>All dates</th>
<th>Last two dates</th>
<th>All dates</th>
<th>Last two dates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990</td>
<td></td>
<td></td>
<td>1991</td>
<td></td>
</tr>
<tr>
<td>Total N</td>
<td>0.813</td>
<td>0.559</td>
<td>0.010</td>
<td>N L H</td>
</tr>
<tr>
<td>Total P</td>
<td>0.001</td>
<td>N L M H</td>
<td>0.001</td>
<td>N L M H</td>
</tr>
<tr>
<td>Total N:P</td>
<td>0.008</td>
<td>H M L N</td>
<td>0.013</td>
<td>H M L N</td>
</tr>
<tr>
<td>Particulate or seston C</td>
<td>&lt;0.001</td>
<td>N L M H</td>
<td>&lt;0.001</td>
<td>N L M H</td>
</tr>
<tr>
<td>Particulate or seston N</td>
<td>0.002</td>
<td>N L M H</td>
<td>0.019</td>
<td>N L M H</td>
</tr>
<tr>
<td>Particulate or seston P</td>
<td>&lt;0.001</td>
<td>N L M H</td>
<td>&lt;0.001</td>
<td>N L M H</td>
</tr>
<tr>
<td>Particulate or seston C:N</td>
<td>0.698</td>
<td>0.808</td>
<td>0.109</td>
<td>0.015 N L H</td>
</tr>
<tr>
<td>Particulate or seston C:P</td>
<td>0.400</td>
<td>0.028 L H M N</td>
<td>0.134</td>
<td>0.031 H L N</td>
</tr>
<tr>
<td>Particulate or seston N:P</td>
<td>0.365</td>
<td>0.217</td>
<td>0.148</td>
<td>0.095 L H N</td>
</tr>
<tr>
<td>Zooplankton C</td>
<td>...</td>
<td>0.534</td>
<td>0.359</td>
<td></td>
</tr>
<tr>
<td>Zooplankton N</td>
<td>...</td>
<td>0.360</td>
<td>0.500</td>
<td></td>
</tr>
<tr>
<td>Zooplankton P</td>
<td>...</td>
<td>0.240</td>
<td>0.315</td>
<td></td>
</tr>
<tr>
<td>Zooplankton C:N</td>
<td>...</td>
<td>0.361</td>
<td>0.953</td>
<td></td>
</tr>
<tr>
<td>Zooplankton C:P</td>
<td>...</td>
<td>0.112</td>
<td>0.671</td>
<td></td>
</tr>
<tr>
<td>Zooplankton N:P</td>
<td>...</td>
<td>0.332</td>
<td>0.894</td>
<td></td>
</tr>
</tbody>
</table>
only account for \( \approx 36\% \) of the final difference in water column TP between the No Fish and Medium Fish treatments (loss = 33.8 \( \mu \)mol P/L; difference between treatments = 92.7 \( \mu \)mol P/L). However, loss of P from fish tissue can account for 84% of the observed final difference in water column TP between the No Fish and High Fish treatments (loss = 79.7 \( \mu \)mol P/L; difference between treatments = 94.9 \( \mu \)mol P/L).

Water column TP concentrations generally declined in all treatments during the experiment in both years (Fig. 4). The observed change in TP in the water column should equal input of P to the water column from fish minus the loss of P through sedimentation and wall growth. Measured loss of P from the water column through sedimentation and wall growth increased with increasing fish biomass (Table 5). When the predicted net change in water column P is estimated as (inputs from fish – sedimentation – wall growth), the P “budgets” are within 12 \( \mu \)mol P/L in Low and Medium Fish treatments and within 30 \( \mu \)mol P/L in High Fish treatment (Table 5). However, the discrepancy in P balance is much greater (124 \( \mu \)mol P/L) for the No Fish treatment (Table 5).

**DISCUSSION**

**Effects of fish on nutrient dynamics**

In these experiments, fish increased the concentrations of N and P in the water column. The proportion
of total N and P present as particulates also tended to be higher in the presence of fish (Figs. 5 and 6). For example, at the end of the 1990 experiment (average of last two dates), particulate N comprised 11.9, 11.8, 16.5, and 18.3% of total N in the No, Low, Medium, and High Fish treatments, respectively. Effects on P were even more pronounced; particulate P comprised 24.6, 30.1, 35.1, and 40.1% of total P in the No, Low, Medium, and High Fish treatments, respectively. Exclusion of fish also shifted the size distribution of particulate nutrients toward larger particles, at least in 1991. At the end of this experiment (average of last two dates), zooplankton constituted 13.4, 10.6, and 8.9% of particulate N and 23.7, 15.1, and 14.0% of particulate P in the No, Low, and High Fish treatments, respectively. Thus, smaller particles (<63 μm) comprised a greater fraction of particulate N and P when fish were present than when fish were absent.

Increased total P associated with high biomass of zooplanktivorous fish agrees with several other studies (e.g., Andersson et al. 1978, Shapiro and Wright 1984, Mazumder et al. 1988, Drenner et al. 1990, Reinertsen et al. 1990, Lazzaro et al. 1992). Higher particulate P concentration and shifts in particulate phosphorus toward smaller particles in the presence of zooplanktivores were also reported by Mazumder et al. (1988), Drenner and Smith (1991) and Lazzaro et al. (1992). In contrast, Drenner and Smith (1991) and Lazzaro et al. (1992) found no effect of fish on total N. Mazumder et al. (1988) do not provide total N data and none of the above studies quantified particulate N size distributions. Based on these previous studies and results
from our experiments, it appears that zooplanktivorous fish consistently increase water column total P and particulate P concentrations, consistently decrease the mean (or median) particle size of particulate P, and have variable effects on total N concentration.

**Food web effects on nutrient recycling and stoichiometry**

Recent research on stoichiometric relationships in planktonic food webs suggests that variation in the species composition of the herbivorous zooplankton assemblage can greatly affect the ratio at which N and P are recycled and incorporated into phytoplankton (Sterner 1990, Andersen and Hessen 1991, Hessen and Lyche 1991, Sterner et al. 1992). Large cladocerans, especially *Daphnia* but also *Holopedium*, tend to have relatively high P and low N contents (percentage of dry mass) in their tissues. In contrast, calanoid copepods tend to have much lower P contents and somewhat higher N contents than large cladocerans, while other taxa such as cyclopoid copepods and *Bosmina* tend to have intermediate P contents (Andersen and Hessen 1991, Hessen and Lyche 1991). Individual species seem to maintain relatively constant nutrient concentrations (and ratios) in their bodies regardless of dietary nutrient content (Sterner and Hessen 1994). Fewer data exist on rotifers, but Makarewicz and Likens (1979) showed that N and P contents of rotifers ( multispecies samples) are similar to that of calanoid copepods. Fish may therefore regulate stoichiometric processes by controlling zooplankton species composition, and it is useful to discuss whether the results of our enclosure

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**Fig. 7.** C, N, and P contents and their ratios in zooplankton in the 1991 experiment.
Table 4. Loss of dry mass, carbon, nitrogen, and phosphorus from fish in the 1990 experiment. For experimental treatments, values are means of three replicate enclosures and the st (in parentheses) represents variation among enclosures. For “lake,” fish values are means of 10 fish collected from Tuesday Lake on 1 August 1990, and the st (in parentheses) represents variation among individual fish. Letters next to values for nutrient content of fish tissue indicate statistical differences among treatments within each nutrient; nutrient contents of lake fish were not included in the statistical analyses.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dry mass of fish (g/enclosure)</th>
<th>Nutrient content of fish tissue (% of dry mass)</th>
<th>Total nutrient release from fish to water column over entire experiment (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Start of experiment</td>
<td>End of experiment</td>
<td>Carbon</td>
</tr>
<tr>
<td>Low fish</td>
<td>0.511 (0.013)</td>
<td>0.453 (0.023)</td>
<td>40.15 (+)</td>
</tr>
<tr>
<td>Medium fish</td>
<td>1.040 (0.009)</td>
<td>0.793 (0.022)</td>
<td>38.78 (+)</td>
</tr>
<tr>
<td>High fish</td>
<td>1.787 (0.028)</td>
<td>1.183 (0.018)</td>
<td>36.99 (+)</td>
</tr>
<tr>
<td>Lake</td>
<td>…+†</td>
<td>…†</td>
<td>42.53 (0.70)</td>
</tr>
</tbody>
</table>

† Ellipses indicate that fish biomass and nutrient release from fish were not quantified in Tuesday Lake.

Experiments agree with predictions of stoichiometry theory. This is especially so because Tuesday Lake fish–zooplankton–phytoplankton interactions have been used as an example of how food web changes may have stoichiometric effects (Elser et al. 1988, Sterner et al. 1992).

Two predictions from stoichiometry theory are relevant to our experiments. The first prediction is that exclusion of zooplanktivorous fish (as in our No Fish treatment) should result in decreased C:P and N:P ratios in the zooplankton fraction, reflecting increased relative abundance of species with high body P concentrations, such as *Daphnia* and *Holopedium*. We found moderate support for this prediction. C:P and N:P ratios in the zooplankton fraction tended to be higher in the No Fish treatment compared to other treatments during dates when *Daphnia* peaked in the No Fish treatment. In addition, the dynamics of zooplankton nutrient ratios in the No Fish treatment seem to track the abundance of *Daphnia*. That is, C:P and N:P ratios decline, and C:N ratio increases, when *Daphnia* comprises a large proportion of zooplankton biomass (17–27 June; see Figs. 2 and 7). However, treatment effects on zooplankton nutrient ratios did not persist over the course of the experiment (Fig. 7), and effects on zooplankton N:P were only marginally significant even during the *Daphnia* peak.

If we had seen a complete shift from calanoid copepods to *Daphnia* upon exclusion of fish, we may have seen stronger effects of fish exclusion on zooplankton nutrient ratios (Sterner et al. 1992). Such differences in taxonomic composition would have maximized among-treatment difference in zooplankton body nutrient ratios. We observed near total exclusion of *Daphnia* in the presence of fish and relative high abundance of *Daphnia* in the absence of fish. However, cyclopoid copepods, *Bosmina*, and/or rotifers comprised significant portions of total zooplankton biomass; calanoid copepods were virtually absent in 1991. In addition, copepod biomass was not significantly affected by fish in 1991 (Table 2). Because zooplankton assemblages in our enclosures contained a substantial proportion of...
taxa with intermediate body P content (and hence N:P ratio), and because some abundant groups (e.g., cyclopooids) were not affected by fish, the differences in zooplankton species composition we observed among treatments were apparently not great enough to be manifested in large differences among treatments in nutrient ratios of the zooplankton pool.

The second relevant prediction of stoichiometry theory is that repeated cycles of ingestion and excretion by contrasting zooplankton assemblages should result in higher seston N:P ratio in the No Fish treatment compared to other treatments. Zooplankton should excrete at higher N:P ratios in the No Fish treatment compared to other treatments, at least when *Daphnia* and *Holopedium* dominate zooplankton biomass in the No Fish treatment. This follows from the premise that these species, having low tissue N:P ratios, also sequester nutrients at a low N:P ratio to maintain nutrient homeostasis. If so, they should recycle nutrients at a relatively high N:P ratio than zooplankton in enclosures with fish (Sterner 1990, Sterner et al. 1992). According to stoichiometry theory, this should lead to higher seston N:P ratios where *Daphnia* and *Holopedium* are abundant, because phytoplankton would rapidly utilize excreted dissolved nutrients, and their cellular chemical composition reflects nutrient supply ratio.

Our results also show some support for this prediction of stoichiometry theory. Particulate C:P ratios in 1990 and seston C:P and N:P ratios in 1991 were higher in the No Fish treatment than other treatments at the end of both experiments. Because phytoplankton likely comprise most of the biomass in these fractions, phytoplankton nutrient contents thus may reflect a higher N:P supply ratio in the No Fish treatment. A result not consistent with stoichiometry theory is that effects on particulate and seston nutrient ratios occurred well after the peak of *Daphnia* abundance, and hence well after the time of maximum among-treatment differences in zooplankton species composition. If the hypothesized stoichiometric processes (Sterner 1990, Sterner et al. 1992) are important, it is somewhat surprising that phytoplankton nutrient ratios responded so late in the experiment. Tuesday Lake phytoplankton are nutrient limited, as evidenced by the particulate C:P and N:P ratios in Fig. 6 (Hecky et al. 1993) and previous research on Tuesday Lake (Elser et al. 1988, Carpenter et al. 1993b), and should rapidly utilize recycled nutrients. Physiological assays on our enclosure phytoplankton also suggested greater P limitation and lesser N limitation in the No Fish enclosures compared to those with fish, consistent with stoichiometry theory (Vanni and Layne 1997). As with effects on zooplankton nutrient content, perhaps we would have seen stronger effects on seston nutrient ratios had we observed larger differences in zooplankton taxonomic composition among treatments.

In Tuesday Lake and other nearby lakes with similar food webs, nutrient recycling by zooplanktivorous fish may be an important source of P for phytoplankton (Schindler et al. 1993). In fact, simulation modeling suggests that in lakes in this area with abundant zooplanktivorous fish (such as Tuesday Lake), fish recycle much more P to phytoplankton than do zooplankton (Schindler et al. 1993). Further evidence for the importance of nutrient recycling by fish derives from additional experiments we conducted in Tuesday Lake, which suggest that nutrient recycling by fish contributed to the phytoplankton community response to fish (Vanni and Layne 1997). Relatively high excretion by fish could possibly lessen the importance of treatment differences in excretion rates and ratios of zooplankton.

Effects of fish on the nutrient content of the zooplankton fraction may have been obscured to a degree by use of a 63-μm mesh to separate zooplankton and seston fractions. While this mesh size is common for zooplankton sampling, it is probably small enough to trap some phytoplankton, particularly large dinoflagellates that often dominate the Tuesday Lake assemblage (Carpenter et al. 1993b, Vanni and Layne 1997). This may have contaminated the zooplankton fraction somewhat. Using a larger mesh would have reduced algal contamination but might have allowed a substantial fraction of the zooplankton assemblage to pass through, especially in treatments in which rotifers and copepod nauplii were abundant. However, inspection of the nutrient ratios of the 1991 “seston” (<63 μm) and “zooplankton” (>63 μm) fractions suggests that algal contamination of the zooplankton fraction may not have been a problem. For example, there is virtually no overlap in C:P or C:N ratios of the two fractions (Figs. 6 and 7). Assuming that the <63 μm fraction is mostly phytoplankton, this suggests there was little phytoplankton contribution to the >63 μm fraction. Furthermore C:P and C:N ratios in our zooplankton fraction are fairly similar to what would be expected in pure zooplankton samples (Andersen and Hessen 1991, Hessen and Lyche 1991), and lower than what would be expected for nutrient-limited phytoplankton (Hecky et al. 1993).

In summary, we found some support for stoichiometry theory, but not as much as expected from model predictions based on stoichiometry of food web components. The relatively moderate effects of fish on seston stoichiometry may have arisen from (1) smaller among-treatment differences in zooplankton assemblages than assumed in stoichiometric models, and (2) nutrient excretion by fish, which may obscure effects of nutrient recycling by zooplankton.

**Phosphorus budgets**

Phosphorus dynamics in enclosures reflect the balance of P leaving the water column through sedimentation and growth of organisms on enclosure walls, and the net release of P from fish (Table 5). Fish lost mass in all enclosures into which they were placed (Table 4). However, relative loss of P from fish was less than
mass loss. While fish lost 11.3, 23.8, and 33.8% of their dry mass in the Low, Medium, and High Fish treatments, they lost only 3.3, 8.5, and 11.8% of their P, respectively. Thus it appears that these fish conserve P under conditions causing loss of mass, and therefore, the body P content of fish can be quite variable within species. This is unlike that of zooplankton P content, which seems to be relatively constant within species regardless of nutritional conditions (Andersen and Hessen 1991). Loss of P from fish cannot account for the higher total P concentrations in enclosures with fish compared to fishless enclosures, at least not in the Low and Medium Fish treatments.

Phosphorus lost to enclosure wall growth and sedimentation of P from the water column both increased with increasing fish biomass (Table 5). The periphyton response is somewhat surprising because northern redbelly dace consume periphyton in Tuesday Lake (Cochran et al. 1988). Mazumder et al. (1989) found that P sedimentation rate was higher in the presence of zooplanktivorous fish than in their absence, consistent with our results. Our sedimentation rates are somewhat low compared to other published estimates. Mean areal sedimentation rates in our enclosures ranged from 7.7 to 9.9 mmol P·m⁻²·d⁻¹ (Table 5), Mazumder et al. (1989) and Baines and Pace (1994) report P sedimentation rates ranging from 24 to 270 mmol P·m⁻²·d⁻¹. However, areal sedimentation rates are probably higher in lakes with deep euphotic zones than in lakes with shallow euphotic zones, such as Tuesday Lake. Thus, it is perhaps more informative to compare the fraction of total P sinking. Mazumder et al. (1989) found that fractional sedimentation rate was lower in enclosures with fish than in fishless enclosures. We calculated fractional P sedimentation rates for our 1990 enclosures, using the second sediment trap interval (25 July–15 August). Mean fractional sedimentation rates declined with increasing fish biomass (0.92, 0.83, 0.59, and 0.66% of initial total P sedimenting per day in the No, Low, Medium, and High Fish treatments, respectively). This is consistent with the results of Mazumder et al. (1989). Furthermore, if we assume that fish increased primary production rates as well as phytoplankton biomass, our results are also consistent with those of Baines and Pace (1994). They found that the percentage of primary production sinking from the water column decreased slightly with increasing water column primary production rate.

It is possible that we underestimated sedimentation rates because our traps were in place for relatively long time periods compared to other studies. This may have allowed for substantial remineralization of P. We chose long time periods because we wished to minimize the resuspension of settled material (on enclosure bottoms) that may occur when traps are lifted. However, even if we underestimated sedimentation rates, this should not affect whether our P budgets balance, as remineralized P should be incorporated into some other ecosystem pool such as water column or wall growth nutrients.

Phosphorus budgets were essentially “balanced” in enclosures with fish, but were imbalanced in fishless enclosures due to an apparent underestimation of P sinks (Table 5). In fact, the imbalance is quite substantial; predicted water column P decline based on sedimentation and wall growth is only 57% of actual water column P decline (Table 4). Reasons for this imbalance are not clear but may involve methods we used to sample large zooplankton and wall growth. In 1990, we sampled particulate P (as combined phytoplankton and zooplankton fractions) and total P with the integrated tube sampler. The tube sampler was probably not efficient at capturing large zooplankton, including larger herbivores such as Daphnia and Holopedium as well as the dipteran larva Chaoborus punctipennis, because some individuals probably can avoid the tube. This would have preferentially underestimated total P concentrations and particulate P concentration in the No Fish treatment because of the relative abundance of large herbivorous zooplankton (Fig. 2) and Chaoborus (Vonder Brink and Vanni 1993). However, inefficient sampling of large herbivores cannot account for the P imbalance in the 1990 No Fish enclosures. For example, if we assume a tissue P concentration of 1.5% of dry mass for Daphnia and Holopedium (Andersen and Hessen 1991), at the end of the experiment large cladocerans would account for <3 μmol P/L. Even if the tube sampler did not catch any large cladocerans, this “unsampled P” would represent a tiny fraction of the 124 μmol P/L imbalance (Table 5).

Chaoborus sampling may present a more important problem because most individuals likely migrated to the bottom of the enclosures during the day (Soranno et al. 1993a) and thus were not captured even by our Schindler trap hauls. We can crudely estimate the potential importance of Chaoborus in our enclosure P budgets, using Chaoborus population densities from Vonder Brink and Vanni (1993) and assuming a mean individual mass of ~150 μg dry mass (Soranno et al. 1993a) and a P content of 0.81% of dry mass (M. J. Vanni, unpublished data). If we also assume a 58% efficiency for our Wisconsin net (Soranno et al. 1993a), daytime estimates of Chaoborus P content are 38.4, 14.9, 5.9, and 0.6 μmol P/L in the No, Low, Medium, and High Fish treatments, respectively. These estimates may be low because they are daytime estimates, but we have no way of knowing how low they may be. However, inclusion of Chaoborus in our P budgets could account for a substantial proportion of “missing P” in the 1990 No Fish enclosures if the tube sampler did not capture any Chaoborus and if actual densities were twice that of daytime estimates, both of which do not seem unlikely. This would yield a Chaoborus P content of 76.9 μg P/L in the No Fish enclosures, a sizeable fraction of the 124 μmol P/L imbalance. Schindler et al. (1993) found that Chaoborus comprised
much more P than either all other zooplankton or fish in a lake nearby Tuesday Lake, in which piscivorous fish were abundant and planktivorous fish scarce. They also estimate (using simulation models) that Chaoborus may recycle more P than either fish or herbivorous zooplankton in this lake. In contrast, in a lake with few piscivores and abundant planktivores, Chaoborus made up an insignificant proportion of P standing stock or excretion (Schindler et al. 1993). Thus, considerable evidence exists that Chaoborus can be important to P budgets when planktivorous fish are scarce, such as in our No Fish enclosures. Emergence of Chaoborus adults from enclosures could also represent a sink for P, but we rarely observed Chaoborus pupae.

\[ \text{P accumulation as wall growth may also have been underestimated in the No Fish enclosures. We observed loosely attached, “hairy” growths of algae (probably Mougeotia) on the No Fish enclosure walls, and these growths may have sloughed off as we removed the plastic strips used to assess periphyton growth. These algal growths were also observed on ropes used to suspend sediment traps, which were not sampled. Since these growths were much more extensive in No Fish enclosures, underestimates of P on enclosure walls would be most severe for this treatment.} \]

**Enclosure effects**

Enclosures such as those we employed are used widely in aquatic ecology, yet debate exists as to whether they adequately simulate lake trophic interactions. While enclosures are generally thought to be adequate for assessing particular mechanisms underlying trophic interactions, their value in predicting whole-lake processes is not certain, given the scale differences between enclosures and lakes (Carpenter and Kitchell 1988, Frost et al. 1988, Cooper and Barmenta 1993). Tuesday Lake has been the site of extensive whole-lake manipulations of piscivorous and planktivorous fish (Carpenter and Kitchell 1993a), and overall our results are similar to the results of these whole-lake experiments. For example, in our experiments fish exclusion resulted in increased dominance of large cladocerans, decreased abundance of smaller zooplankton species, decreased phytoplankton biomass and increased phytoplankton P limitation. These same responses were observed when piscivores were added at the whole-lake scale, which effectively eliminated zooplanktivorous fish (Carpenter et al. 1993a, b, Soranno et al. 1993a, b). Fewer nutrient data are available for comparison. Mean annual water column total P in Tuesday Lake showed virtually no response to fish manipulations (Carpenter et al. 1993a), although seasonal data were not presented. No data are available on the response of total N or particulate nutrients to whole-lake manipulations.

Another means of assessing whether enclosures satisfactorily simulated lake conditions is to compare particular parameters in the lake and enclosures. Enclosures seemed to adequately “track” the dynamics of total phytoplankton biomass and nutrients in the lake. For example, total N and P and the total N:P ratio showed at least as much temporal variation in the lake as in any particular enclosure treatment (Fig. 4). The decline in total P over the course of the experiment was similar in enclosures and the lake. Particulate nutrient concentrations were generally lower in the lake than in enclosures, although differences between the lake and the No Fish treatment were generally small for most of the experiment (Fig. 5).

Enclosure experiments are most likely to adequately represent lake trophic interactions and nutrient dynamics if enclosures (1) adequately reflect the lake’s thermal stratification regime so that, among other things, food-web-mediated effects on sedimentation can be expressed (Bloesch and Bürgi 1989, Sarnelle 1993); (2) encompass the euphotic zone so that grazing effects on light transmittance (via effects on light-absorbing phytoplankton) are realistic (Mazumder et al. 1990, Sarnelle 1992, 1993); and (3) are large enough to include realistic densities of fish, so that impacts of fish are not exaggerated. We are confident that we met both of the first two criteria. Sediment traps were placed in water that was several degrees colder than surface water, indicating a stably stratified water column. Considering the sheltered nature of Tuesday Lake and that turbulence is usually low in enclosures, there was probably little chance for resuspension of settled material. Carpenter et al. (1993b) report an average euphotic zone (depth receiving 1% of surface light intensity) of 3.3 m, which is within =0.5 m of the depth of our enclosures. Furthermore, grazing effects on light transmittance are not evident in Tuesday Lake, because light attenuation by dissolved humic material in this stained lake overwhelms shading effects of phytoplankton (Carpenter et al. 1993b).

The third criterion, that of realistic fish densities, is somewhat more problematic. Our Low Fish densities are lower than those in the lake, so it would seem that we met this criterion in at least this treatment. However, fish lost mass even in this treatment. Thus, even though loss of fish mass cannot explain differences in P dynamics in enclosures with and without fish, we need to consider the consequences of this for nutrient dynamics, since it is unlikely that this loss of mass typically occurs in the lake. The feeding rate of fish may play a large role in regulating the flux of P through fish. Fish body P content will also affect how much P fish excrete (e.g., Kraft 1992), similar to the situation with zooplankton (Sterner 1990). Therefore our data suggest that a fish’s nutritional history will in part determine its P excretion rate. Although fish displayed a net loss of P, the rate at which they excreted P probably would have been higher if they were well fed and thus gained mass, simply because they would have been processing more P. Studies with bluegill and gizzard shad showed that these fish excrete more P when well
fed than when not fed (Mather et al. 1995). It is important to note that net excretion should not necessarily be equated with net P flux through fish. While well-fed fish may excrete more P than nonfeeding fish, well-fed fish may be a net sink for P if most of consumed P is allocated to growth. If most food is obtained from zooplankton in the pelagic zone, fish might be a net sink for P, and thus decrease the overall amount of P available to phytoplankton, at least over seasonal time scales. Conversely, if fish obtain a significant amount of their food from the littoral zone, they may be a net source of P to phytoplankton even if they grow. In this sense they “transport” P to pelagic areas from the littoral zone (Lamarr 1975, Schindler et al. 1996, Vanni 1996). Littoral prey make up a substantial fraction of the diet of red-belly dace in Tuesday Lake (Cochran et al. 1988, Schindler et al. 1996), and most fish in temperate freshwaters eat at least some littoral benthic prey. Simulation models also show that in similar lakes nearby Tuesday Lake, much of the P excreted by “zooplanktivorous” fish (such as red-belly dace) in the pelagic zone is obtained from littoral prey (Schindler et al. 1996). In fact, littoral-pelagic transport of P by these fish seems to be important in regulating phytoplankton productivity (Schindler et al. 1993, 1996). Thus, while loss of mass (and hence P) from fish may be an artifact of isolating the fish from their littoral zone prey, it seems that this may actually decrease the net rate at which fish supply P to phytoplankton.

Overall our results show close agreement with whole-lake manipulations. This suggests that enclosures such as those used in our study can adequately portray the effects of trophic interactions on nutrient dynamics, provided fish are enclosed at realistic densities. Based on our results and those of others, it appears that fish can have significant effects on nutrient dynamics in lakes, probably through many pathways.

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