

Factors influencing nitrogen and phosphorus excretion rates of fish in a shallow lake

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SUMMARY

1. Fish excretion can be an important source of nutrients in aquatic ecosystems. Nitrogen (N) and phosphorus (P) excretion rates are influenced by many factors, including fish diet, fish growth rate and fish size. However, the relative influence of these and other factors on community-level excretion rates of fish is unknown.

2. We used bioenergetics modeling to estimate excretion rates of eight fish species in a shallow, Minnesota (U.S.A.) lake over four months in both 2004 and 2005. Excretion rates of each species were summed for community-level N and P excretion rates, as well as the N : P ratio of excretion. We then used a model-selection approach to assess ability of independent variables to predict excretion rates, and to identify the most parsimonious model for predicting N : P excretion ratios and P and N excretion rates at the community scale. Predictive models were comprised of the independent variables water temperature and average fish density, fish size, fish growth rate, nutrient content of fish and nutrient content of fish diets at the community scale.

3. Fish density and nutrient content of fish diets (either N or P) were the most parsimonious models for predicting both N and P excretion rates, and explained 96% and 92% of the variance in N and P excretion, respectively. Moreover, fish density and nutrient models had 1400-fold more support for predicting N and 21-fold more support for predicting P excretion relative to models based on fish density only. Water temperature, fish size, fish growth rates and nutrient content of fish showed little influence on excretion rates, and none of our independent variables showed a strong relationship with N : P ratios of excretion.

4. Past work has focused on the importance of fish density as a driver of fish excretion rates on a volumetric basis. However, our results indicate that volumetric excretion rates at the community scale will also change substantially in response to changes in relative abundance of fish prey or shifts in relative dominance of planktivores, benthivores, or piscivores. Changes in community-scale excretion rates will have subsequent influences on algal abundance, water clarity, and other ecosystem characteristics.

Keywords: bioenergetics models, fish diet, fish nutrient excretion, shallow lakes, stoichiometry

Introduction

Productivity patterns of lakes and wetlands are strongly driven by nutrient availability and recycling.

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Zooplankton and microbes were historically thought to be the primary recyclers of nutrients (Lehman, 1988; Vadstein *et al.*, 1993), but recent work has indicated fish can have strong influences on nutrient dynamics. Excretion of phosphorus (P) by fish can be equal to or greater than zooplankton (Vanni & Findlay, 1990; Carpenter, Cottingham & Schindler, 1992; Zimmer, Herwig & Laurich, 2006), higher than

catchment loadings (Brabrand, Faafeng & Nilssen, 1990), and able to meet algal demands for P (Zimmer *et al.*, 2006). Thus, even though relative importance of fish likely varies from system to system, an increased understanding of fish-mediated nutrient cycling will increase our understanding of freshwater ecosystems.

Most research on fish excretion of nitrogen (N) and P has focused on a limited number of fish species present in individual ecosystems (Brabrand *et al.*, 1990; Kraft, 1992; Persson, 1997; Schaus *et al.*, 1997; Zimmer *et al.*, 2006) with fewer studies assessing N and P of entire fish communities (Schindler *et al.*, 1993; Vanni *et al.*, 2002). Studies examining individual fish species on a case by case basis have found excretion rates are influenced by water temperature (Schindler & Eby, 1997; Zimmer *et al.*, 2006), type of fish species (summarized by Vanni, 2002), fish body size (Schaus *et al.*, 1997; Vanni *et al.*, 2002), fish growth rate (Kraft, 1992), nutrient content of fish food (Schindler & Eby, 1997), and nutrient content of fish (Vanni *et al.*, 2002). At the population scale, fish density is obviously a strong predictor of N and P excretion (Kraft, 1992; Schaus *et al.*, 1997; Zimmer *et al.*, 2006). However, community-level estimates have rarely been done (*sensu* Schindler *et al.*, 1993; McIntyre *et al.*, 2007), and the relative importance of species- and population-level factors for community-level excretion rates has not been assessed. This represents a major void in our understanding of the role of fishes in aquatic ecosystems, as influences of fish excretion on other ecosystem components (algal abundance, water clarity, etc.) are likely driven by community-level excretion rates, not population or species specific rates. Thus, understanding factors driving variability in community-level excretion rates will increase knowledge of variability in other ecosystem features.

Comprehending community-level excretion of fish may be especially important in shallow-water ecosystems, as excretion levels in these systems can match or exceed external loading levels (Persson, 1997) and supply the majority of algal demand for N and P (Zimmer *et al.*, 2006). Additionally, factors influencing individual-level excretion (diet, water temperature, etc.) and population-level excretion (fish density) can be highly variable in these systems. This is especially true in shallow lakes and wetlands of the Prairie Pothole Region of North America (PPR), a grassland-wetland complex covering approximately 715 000 km² of the US and Canada (Euliss, Wrubleski

& Mushet, 1999). Winterkill in these shallow lakes can cause substantial inter annual variability in fish abundance (Carlson & Berry, 1990) and community composition (K.D. Zimmer, B.R. Herwig and M.A. Hanson, unpublished data). Also, high interannual and intra-annual variability in water temperature can influence reproductive success of fish (Zimmer *et al.*, 2006). Moreover, fish diets in shallow lakes of the PPR can be highly variable (Duffy, 1998; Herwig & Zimmer, In press), likely reflecting the highly variable aquatic invertebrate communities characteristic of these systems (Zimmer, Hanson & Butler, 2000). Overall, factors influencing individual and population-level excretion rates of fish are highly variable in shallow PPR systems, yet to date the relative importance of water temperature, fish diet, fish density and fish community composition on community-level excretion rates of fish has not been assessed.

Here we use bioenergetics modelling to assess relative importance of fish density, nutrient content of fish prey, nutrient content of fish predators, fish growth rate, fish size and water temperature on community-level excretion rates of N, P and N : P ratios of fish in a shallow lake over 2 years. These predictor variables were chosen because previous empirical or theoretical work has indicated each variable can influence excretion rates in individual fish species, but their relative importance for driving excretion rates at the community scale is unknown.

Methods

Study site

We conducted this study in Lake Christina, a large shallow lake (surface area 1620 ha, mean depth 1.5 m) located in the PPR region of western Minnesota (U.S.A.). The lake has a history of alternating between stable states of turbid, algal dominance, and clear submerged macrophyte dominance (Hanson & Butler, 1994). Fathead minnows (*Pimephales promelas* Rafinesque), brook sticklebacks (*Culaea inconstans* Kirtland) and bluegills (*Lepomis macrochirus* Rafinesque) are the common planktivores, black bullheads (*Ictalurus melas* Rafinesque) and carp (*Cyprinus carpio* L.) are the common benthivores, and northern pike (*Esox lucius* L.) are the dominant piscivore. In an attempt to switch the lake from turbid- to clear-water state, the lake was biomanipulated in the autumn of 2003 via aerial

application of rotenone. Despite extensive fish mortality, small numbers of fish survived the treatment. This research was conducted following the biomanipulation to assess how variable community composition, fish abundance and diet variability (driven by availability of prey) influence community-level excretion rates of fish. All of these factors change rapidly following a natural winterkill or rotenone application (which mimics effects of a winterkill) (Zimmer, Hanson & Butler, 2001), providing a unique situation to address our questions.

Fish sampling

We sampled the fish community on a biweekly basis from June through August of both 2004 and 2005, with five sampling dates per year. We randomly selected 12 sampling stations and visited them each sampling date. Fish were sampled with a 30-m beach seine (3 mm bar mesh), stretching from the waters edge to approximately 1 m in depth, and 200 m² were seined per station. Captured fish were identified, counted and densities of individual fish species were expressed as fish ha⁻¹. Total lengths were measured for up to five randomly selected individuals of each species at each station on each date. Lengths were converted to wet weight based on length–weight regressions (Schneider, Laarman & Gowing, 2000). Up to 10 individuals of each species were randomly selected on each date for diet analysis and preserved immediately with 10% formalin. A total of 17 species of fish were collected across all sampling dates. We restricted analyses to eight species of fish that occurred often enough to model: banded killifish (*Fundulus diaphanous* Lesueur), black bullhead, bluegill, brook stickleback, Iowa darter (*Etheostoma exile* Girard), fathead minnow, pumpkinseed (*Lepomis gibbosus* L.) and yellow perch (*Perca flavescens* Mitchill). These species accounted for 97% of all fish captured in the beach seine.

Bioenergetics modelling

Bioenergetics models require information on fish diets, fish growth rates, energy and nutrient contents of fish, energy and nutrient contents of fish prey, water temperatures, and model parameters based on the physiology of each species of fish. We analysed diets from 633 fish, and preserved fish were dissected

and contents from the anterior one-third of the digestive track were removed for analysis. We did not analyse the posterior two-thirds because prey were digested and difficult to identify. Prey items were identified to the lowest feasible taxonomic level, with identification to the genus level for zooplankton, family or order level for macroinvertebrates and species level for fish. Lengths of invertebrate prey were determined using an image analysis system, and average lengths were subsequently converted to average wet weight using published length–weight regressions. Average weights of each prey item were multiplied by number of prey in the diet to determine total mass of each prey in the diet. Results for individual fish were converted to portion of total diet represented by each type of prey. Proportions for each prey type were then averaged across all fish collected on each date for each species of fish. The model input is thus the average proportion of diet represented by each type of prey for each species of fish on each sample day. We also estimated the average P and N content (as per cent of wet mass) and the N : P ratio (mass ratio) of fish prey at the scale of the entire fish community on each sampling date. This was done by taking a weighted average of N, P and N : P ratio of consumed prey on each date, with the nutrient content of each prey consumed on each date weighted by its proportion of the total prey mass consumed on that date across all fish species.

Fish growth rates were estimated as difference in average fish mass between sampling dates for each species. Mass-specific growth rates at the community scale were estimated by multiplying the mass-specific growth of each fish species by its density ha⁻¹, summing these values across species, and then dividing the sum by the total number of fish ha⁻¹. Energy and nutrient contents of fish predators and their prey were taken from the literature. When published values were not available, values for the most closely related species available were used. Water temperature was measured by suspending four data loggers in 1 m of water, 0.5 m off the bottom. These were dispersed throughout the lake in the same areas fish were sampled. Data loggers measured water temperature at 7-min intervals and the average water temperature for each day was used as a model input.

Physiological parameters for the bioenergetics model of each fish species were taken from the

literature. Parameters from Duffy (1998) were used for fathead minnow models, and bluegill and yellow perch parameters were taken from Hanson *et al.* (1997). Published parameters were not available for Iowa darters, pumpkinseed sunfish, banded killifish, brook stickleback, or black bullhead. Thus, we used published parameters for the most closely related species for these fish. Yellow perch parameters (Hanson *et al.*, 1997) were used for Iowa darters, bluegill parameters (Hanson *et al.*, 1997) for pumpkinseeds, California killifish (*Fundulus parvipinnis* Girard) (Madon *et al.*, 2000) for both banded killifish and brook sticklebacks, and flathead catfish (*Pylodictis olivaris* Rafinesque) (Roell & Orth, 1993) for black bullheads. Use of parameters for closely related species may increase error in model estimates (Ney, 1993). However, our focus was assessing the relative influence of numerous variables on P and N excretion at the community scale, not precise estimates of excretion rates for individual species, so influences of 'borrowing' species parameters on our result should be minimized.

Bioenergetics modelling was used to estimate average excretion rates of N and P of each species on the 10 dates where diet data were available. Modelling was performed using Fish Bioenergetics 3.0 (Center for Limnology, University of Wisconsin-Madison, Madison, WI, U.S.A.). A species was modelled only if it was captured on that date. The model estimated average N and P excreted fish⁻¹ day⁻¹ for each species, and this was multiplied by density of each species to estimate population-level rates of N and P excretion. Excretion rates were then summed across all species present on each date to obtain a community-level estimate of N and P excretion ha⁻¹, as well as the community level N : P ratio of excretion. We also estimated the average P and N content (as percent of wet mass) and the N : P ratio (mass ratio) of the fish community on each sampling date. This was done by taking a weighted community-level average of N, P and N : P ratio of fish tissue on each date, with the nutrient content of each species collected on each date weighted by its proportion of total fish density on that date.

Statistical analysis

We had two main objectives in this study. First, we wanted to assess and compare the ability of fish

density, nutrient content of fish prey, nutrient content of fish predators, fish growth rate, fish size, and water temperature (independent variables) to predict community-level excretion rates of N, P and N : P ratios (dependent variables) across the 10 sampling dates. Secondly, we wanted to determine the most parsimonious model for predicting N, P and N : P ratios, with models comprised of varying combinations of the independent variables. We used an information theoretic framework to meet both objectives (Anderson, Burnham & Thompson, 2000). This model-selection approach identifies the most parsimonious model from a set of models; in essence maximizing variance explained using the fewest number of parameters. We selected our independent variables and models *a priori* based on previous studies documenting importance of the variables at the population level.

Fish density is a logical predictor of rates of fish excretion at the community scale, and several studies have documented its importance at the population scale (Schaus *et al.*, 1997; Zimmer *et al.*, 2006). Our other predictor variables have also been shown to potentially have strong influences on nutrient excretion rates or ratios at the population level: fish body size (Brabrand *et al.*, 1990), water temperature (Schaus *et al.*, 1997), fish growth rates (Schindler & Eby, 1997), nutrient content of fish prey (Higgins, Vanni & González, 2006) and fish nutrient content (Vanni *et al.*, 2002). These independent variables can be broadly categorized as physiological variables (body size, water temperature and growth rates) or stoichiometric variables (nutrient content of fish prey and nutrient content of fish predators).

In all cases models were evaluated based on their ability to predict N, P and N : P ratios of excretion at the community scale on the 10 sample dates. Our analysis consisted of four classes of models based on the number of independent variables: none (hereafter referred to as 'base model', this model fits the mean and variance only), one, two and three independent variables. Because the base model simply fits the mean, we use it to assess the parsimony of our more complex models with one–three independent variables. Similar performance between the base model and the more complex models indicates impendent variables used in complex models have poor explanatory power, and likely have little influence on the dependent variables.

N and P excretion rates were analysed separately, and one-variable models consisted of the six independent variables analysed alone: water temperature, average fish size, mass-specific growth rates of fish, fish density, nutrient content of fish (either P or N) and nutrient content of fish diet (either P or N). Analysing one-variable models allowed us to assess the utility of each independent variable as a predictor of nutrient excretion at the community scale, and to also assess whether predictive power and parsimony of single variable models can be improved by use of multi-variable models.

Fish density was hypothesized to be the best overall predictor, so it was paired with the five other independent variables for our two-variable models. Stoichiometry predicts nutrient excretion rates are a function of the overall imbalance in nutrient content between predator and prey, so we also tested a two-variable model consisting of nutrient content of fish and nutrient content of fish diet. Our three-variable models consisted of fish density and fish size (physiological variable) paired with either nutrient content of fish prey or nutrient content of fish predators (stoichiometric variables). We included fish size as the physiological variable because we hypothesized it would be the best physiological variable. Overall, 15 models were tested for their ability to predict both N and P: a base model, six single-variable models, five two-variable models (fish density paired with the five other independent variables), and two three-variable models (fish density + nutrient content of diet + fish size, and fish density + nutrient content of fish + fish size).

We used different models to analyse N : P ratios of excretion. We did not include fish density or water temperature in our analysis of N : P ratios because we did not feel that either variable would influence N : P ratios of excretion. We again fit a base model, and also tested four models with one-independent variable: fish size, fish growth rate, prey N : P ratio and fish N : P ratio. We also tested two models with two-independent variables, fish size + prey N : P and fish size + fish N : P ratio. Thus, seven models were assessed for their ability to predict N : P ratios of excretion, four single-variable models, two two-variable models, and one model with no independent variables.

All independent and dependent variables were log transformed to increase homoscedasticity of residuals.

Multicollinearity among our independent variables could complicate our model selection analysis, so we assessed correlations among our six independent variables across the 10 sample dates. Model selection was performed using JMP 5 (SAS Institute Inc., 2002), and model parsimony was assessed using R^2 , AIC_c , w_i (Akaike weights) and evidence ratios (Anderson *et al.*, 2000). Briefly, models with lower AIC_c scores have greater parsimony, and have an optimized balance of predictive power without being over fit with too many independent variables. AIC_c values can be converted into w_i and evidence ratios, the former is the proportion of support for a given model relative to all other models being considered, and the latter is multiplicative support of the top-ranked model relative to all other models. Thus, an evidence ratio of 100 for the second-best model indicates the top model has 100-fold more support relative to the second-best model. Models with evidence ratios >10 are considered implausible (Anderson *et al.*, 2000). Our approach can be viewed as a sensitivity analysis, as our fundamental question is which input variables for the bioenergetics model (fish size, water temperature, etc.) best explain variance in the model output (fish excretion rates and ratios).

N and P content and N : P ratios of the fish community and fish prey on each date are a function of the relative abundance of individual species on each date. We used principal component analysis (PCA) to summarize major patterns of species composition of both fish diets and fish community composition. We then examined the relationship between species composition and nutrient content of fish diets, and the relationship between species composition and nutrient content of the fish community. Our PCAs were performed on proportions, with the density of each fish species converted to proportion of total number of fish, and total amount of prey consumed at the community level converted to proportion of total prey consumed for each date. Using proportions prevented the PCAs from being driven by differences in fish density and amount of prey consumed, and instead focused on differences in composition. PCA on fish community composition was performed on proportional data of the eight species of modelled fish, while PCA on diet data was performed on proportional data of all 29 types of consumed prey. PCA was performed using Canoco 4.5 (ter Braak & Smilauer, 2002).

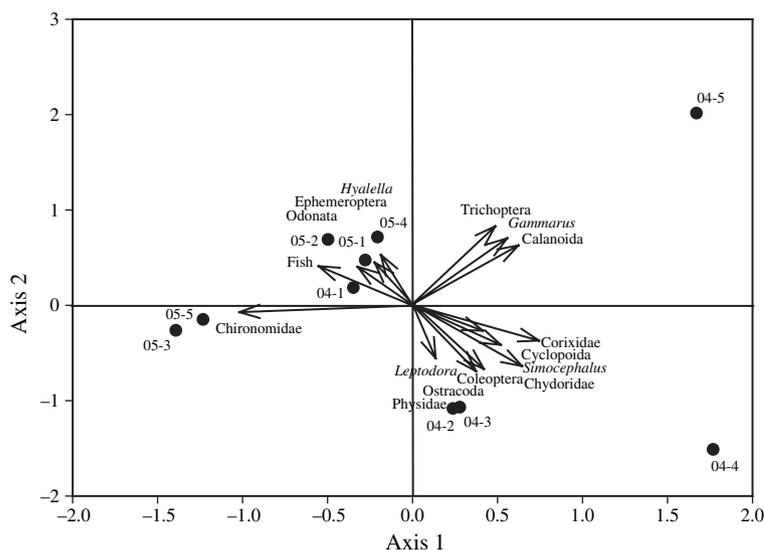
Results

All dependent and independent variables exhibited a wide range of values across the 10 sample dates. P excretion rates ranged from 0.03 to 9.8 g ha⁻¹ day⁻¹, N from 0.2 to 49.3 g ha⁻¹ day⁻¹ and N : P ratios from 5.0 to 24.5 (mass ratios). These excretion rates are much lower than estimates from similar shallow lakes (e.g. Zimmer *et al.*, 2006), likely due to reduced fish densities following the biomanipulation. For our independent variables, water temperature ranged from 20.5 to 28.3 °C, average fish size from 0.07 to 34.2 g (wet mass) and mass-specific growth rates of fish from 0.4 to 71.0 mg g⁻¹ day⁻¹ (wet mass). Nutrient content of fish prey and fish predators at the community scale also varied substantially, although the range of values was higher in fish prey relative to fish consumers. Nutrient content of fish prey ranged from 0.11% to 0.22% P (per cent of wet mass), 1.29–2.98% N and N : P ratios ranged from 11.4 to 16.5. For fish predators, P ranged from 0.25 to 0.32%, N from 1.18 to 1.96% and N:P ratios from 3.7 to 6.3. Species composition of fish prey and the fish community also differed sharply among sample dates. Axis 1 and 2 of the PCA on fish diets explained 84% of the variance in diet composition, with 62% explained by axis 1 alone (Fig. 1). Axis 1 was a gradient of zooplankton versus macroinvertebrate/fish dominated diets, with dates with positive axis 1 scores (2004 sample dates 2–5) having high zooplankton consumption and dates with negative scores (all other sample dates) having high

consumption of fish and macroinvertebrates. On average, diets on dates with positive axis 1 scores averaged 65% macroinvertebrates (by wet mass), 35% zooplankton and 0% fish, while dates with negative axis 1 scores averaged 86% macroinvertebrates, 13% fish and 1% zooplankton. Axis 1 and 2 of the PCA on fish community composition explained 76% of the variance (axis 1 explained 54%), and indicated a gradient of diverse versus simple fish communities (Fig. 2). The date with the lowest PCA axis 1 score (2004 sample date 1) was diverse, with banded killifish (44% of total fish by number), black bullheads (33%), brook sticklebacks (11%) and fathead minnows (11%) all being relatively common. In contrast, the date with the highest score on PCA axis 1 (2004 sample date 3) was dominated by fathead minnows (85%). Sample dates between the high and low PCA scores displayed fish diets or fish community composition 'averaged' between the high and low PCA scores described above. Multicollinearity among our independent variables was low; we detected only three significant pairwise correlations among 29 tests. Significant correlations included body size and growth rate (Pearson's $r = -0.69$, $P = 0.026$), fish P content and water temperature ($r = -0.76$, $P = 0.012$), and diet N : P ratio and growth rate ($r = -0.81$, $P = 0.005$).

As hypothesized, fish density was the single-variable model best supported by the data ($R^2 = 0.69$) for predicting N excretion at the community scale (Table 1). Diet N was the second best single-

Fig. 1 Results of principal component analysis on taxonomic composition of fish diets on 10 sampling dates. Circles are the score for each date, and numbers identify the sampling date as 1–5 in either 2004 or 2005. Arrows represent the species vector for representative taxa used in the analysis (out of 29 total taxa), and point in the direction of increasing relative abundance in fish diets.



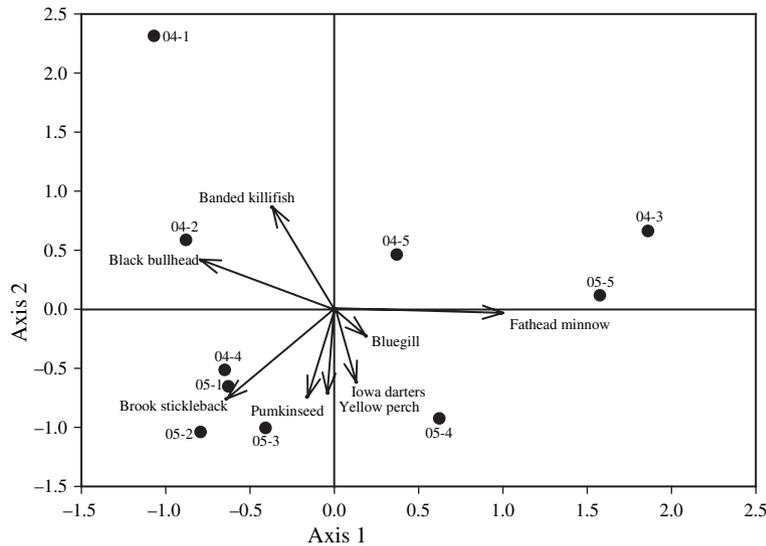


Fig. 2 Results of principal component analysis on species composition of the fish community on 10 sampling dates. Circles are the score for each date, and the numbers identify the sampling date as 1–5 in either 2004 or 2005. Arrows represent the species vector for the eight fish species used in the analysis, and point in the direction of increasing relative abundance of individual fish species.

Model	R^2	K	AIC_c	Δ_i	w_i	Evidence ratio
DENS + DIET N	0.960	4	-17.05	0.00	0.985	
DENS + DIET N + SIZE	0.962	5	-8.47	8.58	0.013	73.1
DENS	0.691	3	-2.49	14.55	0.001	1445.4
DENS + SIZE	0.830	4	-2.46	14.59	0.001	1472.5
DENS + TEMP	0.762	4	0.89	17.94	<0.001	7873.4
DENS + GROW	0.755	4	1.19	18.24	<0.001	9127.4
DIET	0.522	3	1.27	18.32	<0.001	9531.2
DENS + FISH N	0.701	4	3.20	20.24	<0.001	24 895.0
DENS + FISH N + SIZE	0.868	5	4.02	21.07	<0.001	37 602.6
BASE		2	4.97	22.02	<0.001	60 347.8
DIET N + FISH N	0.566	4	6.91	23.96	<0.001	159 659.6
FISH N	0.051	3	8.72	25.77	<0.001	395 087.3
SIZE	0.048	3	8.76	25.80	<0.001	401 295.5
TEMP	0.018	3	9.02	26.07	<0.001	459 048.5
GROW	0.001	3	9.25	26.30	<0.001	513 126.8

Table 1 Performance of 15 models attempting to predict N excretion rates of a fish community. Models are sorted in order of increasing AIC_c values, with smaller AIC_c values indicating more parsimonious models

K is number of parameters in each model (including an intercept and error term), Δ_i is differences in AIC_c values between each model and the most parsimonious model (model with lowest AIC_c value), w_i are Akaike weights and represent weight of evidence (out of 1.00) that each model is the best model in the set, and evidence ratio shows multiplicative improvement of the best model over all other models. The BASE model estimates mean N excretion rate and variance only.

DENS, fish density; DIET N, N content of fish diet; FISH N, nitrogen content of fish predators; TEMP, water temperature; SIZE, fish size; GROW, fish mass-specific growth rates.

variable model ($R^2 = 0.52$), but all other single-variable models performed poorly (all $R^2 < 0.06$). However, the two-variable model fish density + diet N was the best overall model ($R^2 = 0.96$), and was 1445-fold better supported compared with the fish density model, and 73-fold better supported than the second best overall model (fish density + diet N +

fish size). The fish density + diet N model was a substantial improvement over the fish density model because four dates (2004 sample dates 2–5) had substantially lower N excretion rates than would be expected based solely on fish density, but had diets lower in N relative to the other six sampling dates (Fig. 3). These four dates with low N excretion rates

Table 2 Performance of 15 models attempting to predict P excretion rates of a fish community. Models are sorted in order of increasing AIC_c values, with smaller AIC_c values indicating more parsimonious models. Acronyms for model variables, statistical symbols, and terms are defined in Table 1

Model	R^2	K	AIC_c	Δ_i	w_i	Evidence ratio
DENS + DIET P	0.924	4	-9.13	0.00	0.878	
DENS	0.747	3	-3.07	6.06	0.042	20.7
DENS + SIZE	0.858	4	-2.83	6.30	0.038	23.4
DENS + DIET P + SIZE	0.935	5	-1.69	7.44	0.021	41.3
DENS + GROW	0.823	4	-0.67	8.46	0.013	68.7
DENS + FISH P	0.768	4	2.07	11.20	<0.001	270.7
DENS + TEMP	0.757	4	2.44	11.57	<0.001	326.0
DIET P + FISH P	0.689	4	4.97	14.11	<0.001	1156.8
DIET P	0.426	3	5.11	14.25	<0.001	1241.0
DENS + FISH P + SIZE	0.866	5	5.54	14.67	<0.001	1533.0
BASE		2	6.38	15.51	<0.001	2334.2
FISH P	0.210	3	8.30	17.43	<0.001	6107.9
SIZE	0.074	3	9.90	19.03	<0.001	13 572.9
GROW	0.001	3	10.65	19.78	<0.001	19 767.3
TEMP	0.001	3	10.66	19.80	<0.001	19 886.7

were the same dates that had positive scores on axis 1 of the PCA on fish diets, indicating dates with lower than expected N excretion occurred when fish diets were high in zooplankton and low in fish and macroinvertebrates (Fig. 1).

Results for P excretion were similar to those for N, with fish density again being the best single-variable model ($R^2 = 0.75$), followed by diet P ($R^2 = 0.43$), with all other single-variable models poorly supported

($R^2 < 0.08$) (Table 2). The two variable model fish density + diet P was also best-supported for P excretion ($R^2 = 0.92$), and had 21-fold more support than the fish density model, and 23-fold more support than the third best overall model of fish density + fish size ($R^2 = 0.86$). The fish density + diet P model was again an improvement over the fish density model because sample dates with lower than expected P excretion rates based on fish

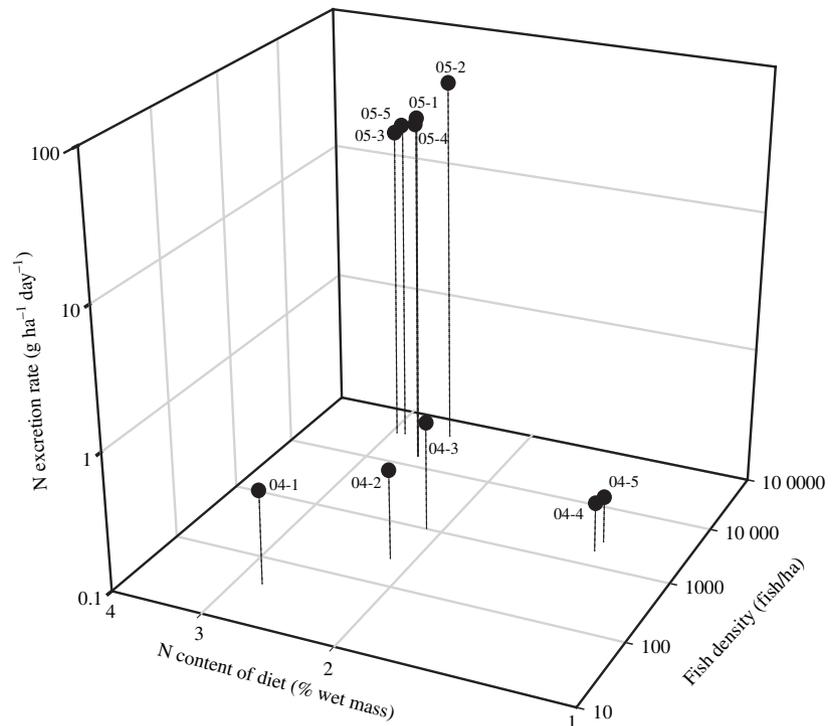


Fig. 3 Relationship between N excretion rates and (i) N content of fish diets (ii) fish density. Diet N content and fish density comprised the most parsimonious model for predicting N excretion rates, and together explained 96% of the variance in N excretion. Numbers associated with each date identify the sampling date as 1–5 in either 2004 or 2005, and are presented for comparison to the scores for each date in Figs 1 and 2.

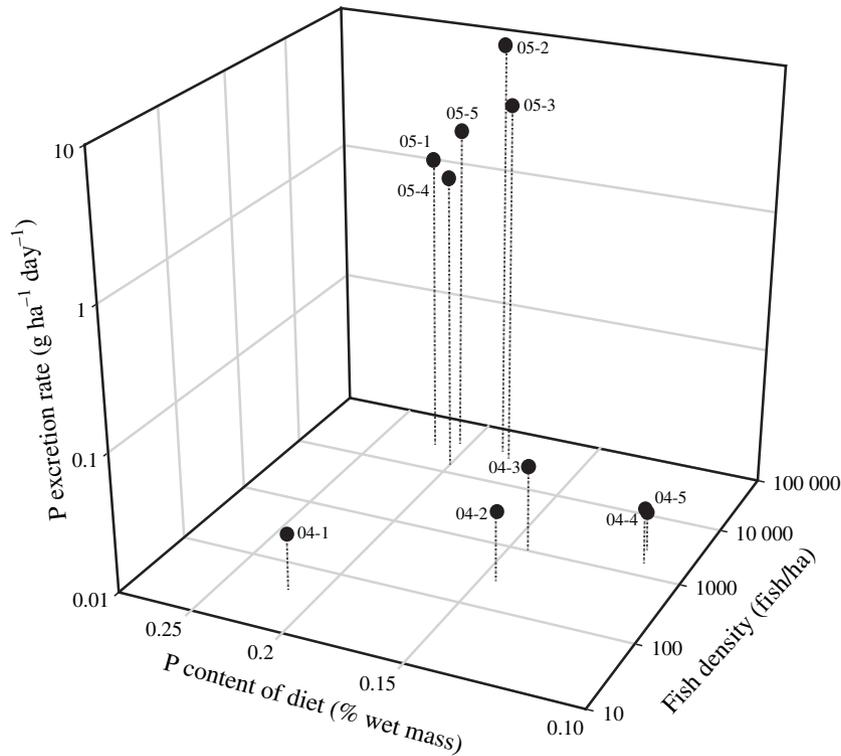


Fig. 4 Relationship between P excretion rates and (i) P content of fish diets and (ii) fish density. Diet P content and fish density comprised the most parsimonious model for predicting P excretion rates, and together explained 92% of variance in P excretion. Numbers associated with each date identify the sampling date 1–5 in either 2004 or 2005, and are presented for comparison to the scores for each date in Figs 1 and 2.

density had diets lower in P relative to other dates (Fig. 4). These sample dates with low P in diets (2004 sample dates 2–5) again corresponded to sample dates where fish diets were dominated by zooplankton (Fig. 1). The fish density + diet nutrient models were substantial improvements over the base model for both N and P excretion rates, with the fish density + diet N 60 000-fold better supported than the base N model, and the fish density + diet P 2000-fold better supported over the base P model. Overall, these results show strong support for fish density and nutrient content of fish diets being the most parsimonious model for predicting both N and P excretion from fish, and this model is a substantial improvement over use of fish density alone.

Results for N : P ratios of excretion differed from those obtained for N and P excretion. Overall model performance declined, with only one model having an R^2 value >0.13 , and the base model had the strongest support (Table 3). Diet N : P ratio had the strongest support among all models, but explained just 12% of the variance, and was fourfold less parsimonious relative to the base model (Fig. 5). Diet N : P + fish size explained the most variance ($R^2 = 0.43$), but the large AIC_c value relative to other models indicated it was over parameterized. The base model being best supported indicates N : P ratios of excretion showed little relationship with our independent variables, and that parsimonious models were not included in our analysis.

Model	R^2	K	AIC_c	Δ_i	w_i	Evidence ratio
BASE		2	-24.38	0.00	0.564	
DIET N : P	0.124	3	-21.42	2.96	0.128	4.4
SIZE	0.095	3	-21.10	3.28	0.109	5.2
FISH N : P	0.102	3	-20.22	4.16	0.070	8.0
GROW	0.006	3	-20.16	4.22	0.068	8.3
DIET N : P + SIZE	0.428	4	-19.68	4.70	0.054	10.5
FISH N : P + SIZE	0.100	4	-15.15	9.24	0.006	101.4

Table 3 Performance of seven models attempting to predict N : P excretion ratios of a fish community. Models are sorted in order of increasing AIC_c values, with smaller AIC_c values indicating more parsimonious models. Acronyms for model variables, statistical symbols, and terms are defined in Table 1

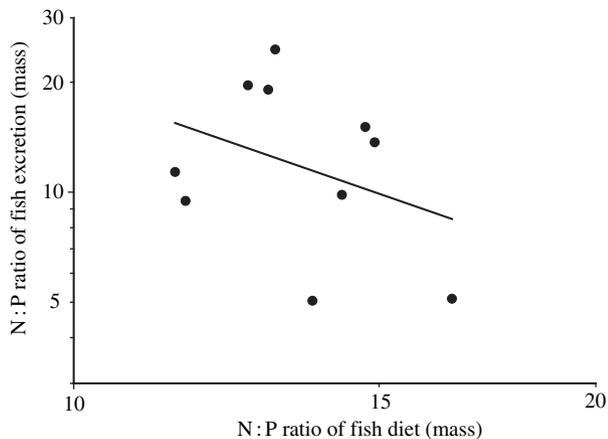


Fig. 5 Relationship between N : P ratio of fish excretion and N : P ratio of fish diets. Diet N : P ratio was the best model utilizing independent variables, but was not as supported as the base model fitting the mean N : P only, and explained only 12% of the variance.

Discussion

Our study indicates that fish size, fish growth rates, and N : P ratios of fish prey and predators have little influence on N : P ratios of fish excretion at the community scale. In contrast, fish density + diet nutrients (either N or P) comprise the most parsimonious model for predicting both N and P excretion rates of fish communities, and this model represents a substantial improvement over models using fish density only. Moreover, fish density + diet nutrients explained a substantial amount of variance in both N and P excretion rates, despite wide variance in other potentially influential variables such as nutrient content of the fish community, water temperature, fish growth rates and average fish size. This indicates that, at the community scale, fish density, followed by nutrient content of fish prey, has the greatest influence on N and P excretion by fish, with water temperature, fish size and nutrient content of fish far less influential. From a practical standpoint, this suggests researchers should prioritize accurate density and diet estimates in order to increase their overall accuracy on estimating community-level excretion rates of fish.

Our results should not be interpreted as evidence that water temperature, fish size, fish growth rates and nutrient content of fish have little or no influence on N and P excretion rates of fish. Numerous studies have shown that these variables have strong influen-

ces on individual species of fish [water temperature and fish size (Zimmer *et al.*, 2006), body size and nutrient content of fish (Vanni *et al.*, 2002), fish growth rates (Kraft, 1992)]. However, our results suggest the influence of these variables declines at the community scale, with only fish density and nutrient content of prey remaining as strong predictors of excretion rates. One possible explanation for our result is the range and variance of values for fish density and nutrient content of prey was much greater than values observed for water temperature, fish growth rates, fish size and nutrient content of fish. Most of these independent variables were measured on different scales, making comparisons of range and variance problematic. Water temperature, however, ranged from 21 to 28 °C, likely representing the normal range for summer months for north-temperate shallow lakes of North America. This suggests the range observed in this study did not bias the influence of water temperature. A more likely mechanism minimizing influences of water temperature at the community scale is variable physiological responses to water temperature across our modelled species. Consumption and metabolic rates relative to water temperature vary sharply among individual fish species (summarized in Hanson *et al.*, 1997). Thus, it is possible that as water temperatures increased in this study, excretion rates of some modelled species increased while others decreased. The net result would be no relationship between water temperature and excretion rates at the community scale, despite strong temperature effects documented at the population scale. It is worth noting that temperature may become more important in north temperate lakes when examined at the scale of an entire year, because of the much larger variance in water temperatures.

It also seems unlikely the poor explanatory power of average fish size and mass specific-growth rates of fish are due to limited range and variance, as the former varied 488-fold and the later 178-fold across the 10 sample dates. Despite large variance, however, mean fish sizes were skewed towards juvenile, small-bodied individuals for many of our modelled species. This was likely due to the biomanipulation the lake underwent prior to sampling, which killed off a substantial number of the large-bodied individuals. Our largest mean fish size at the community scale was 34 g (wet mass), which is below the typical weight of adult bluegill, pumpkinseed, yellow perch and black

bullhead, but higher than adult weights for fathead minnow, brook stickleback, banded killifish and Iowa darter (K.D. Zimmer, B.R. Herwig and M.A. Hanson, unpublished data). Thus, explanatory power of body size might increase if we had captured more adult individuals from our larger species. Given the numerical dominance of small-bodied fish on at least a seasonal basis (Kraft, 1992; Romare *et al.*, 2003; Zimmer *et al.*, 2006), it is unclear how much community-scale excretion rates would be altered by presence of a limited number of larger fish. Similarly, our wide range of values for fish growth rates were skewed towards small-bodied, fast growing fish, and contained few large fish exhibiting little or no growth. It is possible that fish growth rates become more important as the community includes older, slow growing fish. However, the numerical dominance of small-bodied fish at the community scale again seems likely to minimize the influence of larger fish on community-scale rates of fish excretion.

Our results were similar to Torres & Vanni (2007), in that excretion rates showed a stronger relationship with nutrient content of prey relative to nutrient content of fish. The variance and range of observed values for nutrients in prey versus nutrients in fish at the community scale is a likely explanation for the strong predictive power of diet nutrients relative to fish nutrients. P content of individual prey taxa ranged from 0.096% to 0.32% (wet mass), while P concentrations in fish species ranged from 0.20% to 0.41%. Thus, distribution of P concentrations in fish consumers was higher than their invertebrate and fish prey, but predators and prey both exhibited a range of approximately 0.21% in P concentrations. Ranges of N concentrations were also very similar between fish prey and fish consumers, spanning a range of 1.6% for the former and 1.1% for the later. Despite similar ranges of P and N concentrations in fish predators and fish prey when examined at the scale of individual taxa, the discrepancy increases when scaled up to community-level averages. Across the 10 sample dates, P content of fish diets at the community scale ranged from 0.11% to 0.22%, while P contents of fish predators ranged from 0.25% to 0.31% only. In a similar manner, N content of fish diets varied from 1.3% to 3.0% and fish predators from 1.2% to 2.0%. Thus, the much larger range of diet P and N content likely results in much stronger influences on excretion rates relative to nutrient content of fish at the

community scale. Note that species composition of the fish community may have strong influences on community-level excretion rates if compositional changes involve shifts among planktivores, benthivores and piscivores. However, even in this case, influences of the fish community on community-level excretion rates would likely be driven by nutrient content of their prey, not by nutrient content of the fish themselves. Our results also suggest a positive relationship between species richness of fish and stability of community-level excretion rates of fish. Abundance of individual species was highly variable through time in our study, but presence of multiple species kept fish nutrient content relatively constant at the community scale and minimized its influence on community-level excretion rates. Thus, variance in excretion rates may increase as species richness and diversity of fish declines, with subsequent effects on stability of other ecosystem characteristics.

The much larger difference in nutrient content of fish prey relative to fish at the community scale is likely driven by major shifts in diet that are consistent across all fish species. Dietary changes in our modelled fish exhibited a strong dichotomy between consuming high numbers of zooplankton versus high numbers of macroinvertebrates and fish. This dietary shift also represent a major shift in nutrient content of prey, with zooplankton (excluding P in *Daphnia*) generally less nutrient rich relative to macroinvertebrates and fish (see summary in Schindler & Eby, 1997). In contrast, the observed shifts in species composition of the fish community among dates did not result in major changes in nutrient contents of fish at the community scale. Thus, unless changes in species composition of fish communities coincides with a change in nutrient content of fish at the community scale, nutrient content of fish may have weak influences on excretion at the community scale. In contrast, most species of fish have extremely flexible diets, and temporal and ontogenetic dietary shifts between consumption of zooplankton, macroinvertebrates, and fish are common (Persson, 1997; Herwig & Zimmer, In press). Thus, predictable shifts in prey types and associated nutrient content of prey may result in dietary nutrient content being a more consistent influence on community-level excretion rates relative to nutrient content of predators.

In contrast to results for N and P excretion rates, none of our models for N : P excretion ratios were

strongly supported by the data. The model with the strongest support showed a weak negative relationship between N : P ratio of prey and N : P ratio of excretion, opposite the pattern predicted from stoichiometric theory (Sterner & Elser, 2002). However, all of our models had less support than our base model fitting a mean N : P ratio and variance only, providing strong evidence that none of our independent variables had strong influences on N : P ratios of excretion at the community scale. These community-scale results are in contrast to population-level studies on individual fish species, where N : P ratios of excretion have been shown to be influenced by body size (Schaus *et al.*, 1997; Vanni *et al.*, 2002), N : P ratios of fish (Vanni *et al.*, 2002) and fish growth rates (Kraft, 1992). Our inability to detect these influences at the community scale might be due to differential responses of individual fish species to our independent variables. If N : P ratios increase in some species relative to a change in a given independent variable, while ratios decrease in other fish species, the two effects might cancel out and effects at the community scale will be muted. Whatever the mechanism, we found no support for body size, growth rate, or nutrient content of predator and prey driving N : P ratios of fish excretion at the community scale.

The greatest sources of uncertainty in this study are our limited sample size, collection of samples from one lake and 'borrowing' species parameters for our bioenergetics models. Confidence in results from any study always increases with increasing sample size, but our analysis was restricted to 10 sample dates for logistical reasons. However, our results showed overwhelming support for the importance of fish density and nutrient content of fish diets as drivers of fish excretion rates, increasing the credibility of our results and conclusions. Our study was also limited to a single lake again for logistical reasons, but we feel the variability in species composition of the fish community, fish diets, fish density, and other factors increase its applicability beyond our study system. Lastly, using parameters from closely related species undoubtedly increased bias and error in our estimates of excretion (Ney, 1993; Zimmer *et al.*, 2006). We feel this bias should have minimal influences on our results and conclusions, because our focus was on variables driving community-level excretion rates, not on the specific excretion rates of the species themselves. Thus, our results should be robust, unless

borrowing species parameters influenced relationships between nutrient excretion and water temperature, body size, growth rates, nutrient content of prey and nutrient content of predators in substantially different ways.

Several studies have shown that excretion from fish can be an important source of nutrients in aquatic ecosystems (Persson, 1997; Schaus *et al.*, 1997) and it can influence phytoplankton abundance and community composition (Vanni & Layne, 1997). Our results provide evidence that fish density and nutrient content of fish prey have strong influences on N and P excretion rates of fish communities in shallow lakes, while effects of water temperature, fish size, fish growth rates and fish nutrient content are minimal. This indicates that variance in relative abundance of fish prey and shifts in abundance of functional feeding groups of fish may cause substantial variance in fish excretion rates in shallow lakes. Variance in fish excretion rates, in turn, will potentially influence other ecosystem properties of shallow lakes, including algal primary production, water clarity and abundance of submerged macrophytes (Zimmer *et al.*, 2006).

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