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CARBON, NITROGEN, AND PHOSPHORUS STOICHIOMETRY OF CYPRINID FISHES

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Abstract. We investigated the carbon, nitrogen, and phosphorus levels in whole fish and gut samples of several species of cyprinids, relating our findings to nutrient flux models. Some differences in whole-fish nutrient content across species, lakes, and seasons, as well as differences across fish length and mass, were found. N and P contents were highest in fathead minnows and lowest in pearl dace, with northern redbelly dace and finescale dace intermediate. Larger fish had higher percent C and lower percent N and P. However, all differences in whole fish C, N, and P chemistry were small. Cyprinids had the following mean composition: carbon, 46%; nitrogen, 9.7%; and phosphorus, 1.5%. The cyprinid molar C:N:P ratio was 242:16:1. These values make cyprinids relatively low in phosphorus compared to other fish that have been previously studied, especially members of the Percidae and Centrarchidae. Gut contents were lower in N and P than the whole fish, and C:N and C:P ratios were correspondingly higher in gut contents than in the whole fish. Thus, minnows must concentrate both of these nutrients within their biomass compared to what they eat. The N:P ratio of minnows and minnow gut contents had nearly identical means. All chemical variables showed lower variation in the fish than in the gut contents, supporting a homeostatic model of nutrient flux. Stable nitrogen isotope analysis found that minnows were ~3‰ (parts per thousand) heavier than their gut contents, providing evidence that gut contents analyzed were derived mainly from ingested material. A homeostatic nutrient model appears to be an appropriate one for fish.

Key words: carbon; cyprinids; fish, nutrient content; minnow; ^{15}N; nitrogen; nutrient; phosphorus.

INTRODUCTION

That fish participate directly in nutrient cycles has been known for many years (Lamarra 1975); however, their precise roles have recently begun to be clarified (Vanni 1996). Some studies suggest that fish are unimportant in P fluxes in lakes (Kitchell et al. 1975, Nakashima and Leggett 1980*b*), but others suggest that fish may be among the most important sources of nutrients to phytoplankton (Brabrand et al. 1990, Vanni and Findlay 1990, Carpenter et al. 1992, Kraft 1992, Schindler et al. 1993). Their high mobility means that fish transportation of nutrients from substrates to open waters can be significant (Schindler et al. 1996). Besides being important to understanding nutrient processing itself, nutrient release by fish is important from the standpoint of trophic theory. A direct flux of nutrients from fish to algae can complicate the interpretation of trophic cascades (Vanni and Layne 1997, Vanni et al. 1997), because a positive relationship between zooplanktivorous fish and algae (Brett and Goldman 1996) might be a result of fish-supplied nutrients, rather than from grazing.

Owing to the practical difficulty of directly measuring the flux of nutrients in and out of fish in natural systems, work has emphasized the use of mass-balance models, some of which are multiple currency, and are based on fish bioenergetics (Kraft 1992, Vanni 1996), and some of which are ecosystem models of single nutrients (Carpenter et al. 1992, He et al. 1993, Schindler et al. 1996). Data that are basic to multiple currency models include the elemental composition of the fish as well as the composition of the food they eat, together with parameters of assimilation efficiency and respiration. Improvements in the information used to construct mass-balance models can be expected to improve model output, and hence help clarify the role of fish in limnological nutrient cycles.

Nutrient flux from resources to consumers and then to waste products can be thought of as a chemical reaction wherein mass must balance. The effect of preservation of stoichiometric balance among consumers, resources, and nutrient recycling has been examined from the viewpoint of homeostatic regulation of the composition of the consumer. When the chemical composition of a consumer is more tightly constrained than the composition of its resources, unstable equilibria can result (Sterner 1990, Andersen 1997). Under consumer homeostasis, the ratio X:Y of two chemical substances recycled by the consumer will be exaggerated compared to the ratio it ingests (Olsen et al. 1986, Urabe 1993). These constraints impose boundary conditions, simplifying the multidimensional system of equations containing multiple species and multiple nutrients

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(Kooijman 1995). Data essential to applying stoichiometric theory include (1) the nutrient content of the consumer, (2) the degree of homeostasis of a consumer's nutrient content, and (3) the nutrient content of consumed resources.

There are many reports of the content of various chemicals and biochemicals in different fishes (Love 1970, 1980), including N and P (McCay et al. 1936, Nakashima and Leggett 1980*a*, *b*, Penczak 1985, Penczak et al. 1985, Lall 1991, Gerking 1995). Allometric relationships have not often been examined, but one study on bluegills (*Lepomis macrochirus*) (Davis and Boyd 1978) reported decreasing percentages of N and increasing percentages of P in larger fish. Hence, the N:P ratio declined with fish size. Nevertheless, a good idea of the range of variation of nutrients in fish, or its taxonomic, allometric, or ecological correlates is still mostly lacking.

The general mass balance for nutrients in fish would encompass terms for ingestion, absorption, growth, reproduction, egestion, and excretion. It is conceivable that homeostatic regulation would occur either in the adjustment of assimilation rates or in the adjustment of metabolic rates and subsequent excretion of soluble end products, or both. This difference in mechanism of nutrient balance is important because excreted nutrients are in immediately available chemical forms whereas egested nutrients must undergo further processing before algal uptake, and may be more important to benthic than pelagic processing.

Some conceptual models of nutrient processing are presented in Fig. 1. If production efficiencies (growth/ingestion) of individual nutrients are constant (a ''passive'' model), the X:Y ratio released by a consumer would be a constant multiple of the X:Y ratio ingested (Fig. 1A). Note that organisms such as these would have variable chemical composition as a function of the nutrient status of their ingesta. This passive model is probably not a good one for many metazoan consumers, which tend to maintain elemental homeostasis. An active maintenance of the homeostasis in the consumer X:Y ratio requires changes in production efficiency with changed X:Y ingested, as in the right figure (Fig. 1B). Both of the lines in Fig. 1B include an equilibrium point where X:Y released equals X:Y ingested. Stable maintenance of the X:Y ratio in the consumer's biomass requires the steeper slope (line ''1''). Here, when the X:Y ingested is larger than the X:Y of the consumer, the X:Y ratio released to the environment is higher than the X:Y ratio ingested. In contrast, the X:Y released to the environment is lower than the X:Y ingested when the X:Y ingested is less than the X:Y of the consumer.

Digestion in teleost fish has been reviewed several times (Barringon 1957, Kapoor et al. 1975, Fänge and Grove 1979). In most teleosts, digestion begins in the low pH stomach (Weatherley and Gill 1987) but takes place mostly in the alkaline intestine. In cyprinids and some other teleost families, however, a true stomach is lacking, and so is its associated low pH digestive processes. In their place is a wide region extention of the foregut, referred to as the ''anterior bulb'' (Hofer 1991). Here, we compare the chemical content of the region from the esophagus to the anterior bulb (foregut) to the length from the intestine to the anus (hindgut). The foregut as defined here should be composed of ingesta and fish-derived material including digestive enzymes. Some limited amount of absorption might take place in the foregut, but most absorption should occur in the hindgut as defined here. In contrast, the matter in the fish hindgut should consist of remains of material that has been at least partially absorbed together with fish-derived enzymes and other gut material derived from the fish.

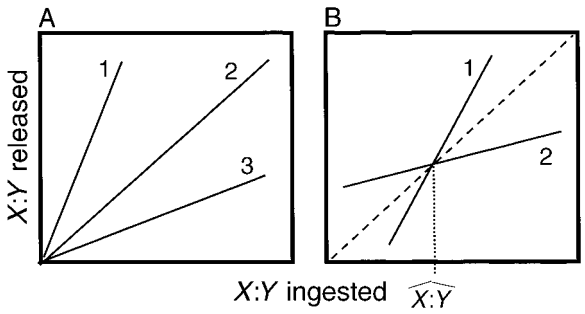

FIG. 1. Hypothetical patterns for nutrient release compared to nutrient ingested under (A) passive or (B) active models. Homeostatic regulation of X:Y can occur only via pattern 1 in panel (B). Ratios (X:Y) can represent either the percentage of mass made up by a single substance (e.g., %P) or the ratio of two chemical substances (e.g., C:P or P:C). In the case of constant assimilation efficiencies for X and Y across all chemical compositions, straight lines intersecting the origin result (A). In (A, 1), assimilation efficiency of Y exceeds assimilation efficiency of X; in (A, 2) the two efficiencies are equal (slope = 1); and in (A, 3) the assimilation efficiency of X is greater than Y. On the other hand, when assimilation efficiency is adjusted according to the composition of the food, nonzero intercepts (B) or perhaps curvilinear relationships (not shown) are expected. In (B, 1) adjustment results in homeostatic regulation; and in (B, 2) adjustment magnifies the composition of the food (not likely). Intersections with the 1:1 line are equilibrium X:Y ratios for the consumer.

In this study, we examined the nutrient content of four species of cyprinid fishes, along with their fore- and hindgut contents. We wished to see how variable the C:N:P ratios of these fish were, and what the correlates of that variability were—specifically, if there were relationships between cyprinid chemistry and fish size, species, lake, or season. Furthermore, we related the nutrient composition of the partially digested matter in the hindgut to the material in the foregut to see if there was evidence for homeostatic regulation by altered digestion. In addition, we will make use of stable isotopes of C and N in this paper to help resolve the C and N balance.

Study Site and Methods

Minnows were collected from several sites within the Experimental Lakes Area (ELA) in northwest Ontario, Canada (Johnson and Vallentyne 1971). The lakes differed considerably in their trophic status. L110, L114, and L226 are naturally oligotrophic lakes, which experience a high N:P loading regime (Hecky et al. 1993), while L227 has been experimentally fertilized with several regimes of N (1970–1988) and P (1970–present) (Findlay et al. 1994), thus maintaining highly eutrophic conditions, including dense cyanobacteria blooms, for >25 yr (Hecky et al. 1994). During the first year of data collection for this study (1992), both lakes studied (L110 and L227) lacked piscivores, and thus the minnows could be considered the uppermost trophic level. In spring of 1993, piscivorous northern pike (*Esox lucius*) were added to both lakes. Catch per unit effort estimates of cyprinid abundance subsequently declined in the following years (George 1994, Elser et al. 1998).

The four species of minnows we encountered were: northern redbelly dace (*Phoxinus eos*) (NRBD), finescale dace (*Phoxinus neogaeus*) (FS), pearl dace (*Margariscus margarita*) (PD), and fathead minnows (*Pimphales promelas*) (FH). These species are small-bodied, cold water species that are generalist feeders on planktivorous zooplankton and benthic organisms (McPhail and Lindsey 1970, Scott and Crossman 1973). They breed primarily in the spring and are often observed in the shallow areas of the lakes.

Fish were collected with either a winged fyke net or commercial minnow traps. Fyke nets were deployed in 1–2 m water depth, and the minnow traps were placed either in offshore or littoral locations. Sampling devices were deployed once per month for 4–5 d and inspected every 24 (or rarely 48) h. For collections involving only whole fish, traps were baited with bread. Fish were frozen within 3 h of collection and were stored frozen. Samples were subsequently lumped into two seasons, May–June and July–August. Collections for gut analysis were made on all four lakes; for these, minnow traps were deployed unbaited, and captured fish remained in the trap for <2 h.

Individuals for whole fish analysis were collected in 1992 from L110 (oligotrophic) and L227 (eutrophic). For analysis of whole fish, first the total length (L, mm) was measured from the tip of the snout to the end of the relaxed caudal fin. Fish were chosen out of the large sample to include a wide distribution of lengths of all four species. Individuals were then freeze-dried for a minimum of 12 h and dry mass (g) was then measured (±1 mg). Fish were then homogenized into a fine powder using a mortar and pestle. For P analysis, 7–10 mg of fish powder was weighed (±1 μg) and ashed at 500°C for a minimum of 4 h. The sample was then reweighed to determine the mass of ash. A subsample of ash (0.1–0.8 mg) was then acid-hydrolyzed with 25 mL of 0.3 N HNO_3. The resulting solution was brought up to 50 mL with distilled water, titrated to pH 7 with NaOH, and analyzed for P using the acid-molybdate method (Strickland and Parsons 1972). This method for determination of P is very similar to the one used by Davis and Boyd (1978). For C and N analysis, a second subsample (1–2 mg) of fish powder was analyzed using a Perkin-Elmer 2400 CHN elemental analyzer.

Table 1. Numbers of minnows of each species obtained from the four study lakes and analyzed for gut chemistry.

Location	FH	NRBD	FS	PD	Total
L110	9	10	2	0	21
L114	12	24	0	0	36
L226	0	27	0	7	34
L227	24	18	0	0	42
Totals	45	79	2	7	133

Note: FH = fathead minnows, NRBD = northern red belly dace, FS = finescale dace, PD = pearl dace.

Whole fish chemistry results including N content, P content, C content (each as percentage of dry mass) as well as the resulting molar ratios were observed to be approximately normally distributed, and they were analyzed by three-way, Type III ANCOVA with fixed effects. Lake, season, and species were considered main effects, and total length and mass were covariates. Multiple comparisons were performed by LSD with significance level of 0.05. Note that though both nutrient content and fish mass are based upon a measurement of mass, the measurements are not the same: in one it is a subsample, and in the other it is the whole fish. For that reason, autocorrelation of experimental error is not a problem.

For gut content analysis, fish were sampled in all four of the study lakes in 1993. A sample of 133 fish was selected from the larger collections to attempt to include a wide dispersion of the four species across the different sample sites (Table 1). Nevertheless, FS and PD were poorly represented. Fish were thawed and then the entire GI tract from mouth to anus was dissected out. Everything from the beginning of the esophagus to the posterior of the anterior bulb was considered ''foregut.'' Contents from the anterior bulb to the anus were considered ''hindgut.'' Fore- and hindguts were flushed using a syringe and distilled water. Gut contents were freeze-dried and weighed. Subsamples were then analyzed for C and N with the CHN analyzer or for P by persulfate digestion followed by acid-molybdate determination of phosphate (Strickland and Parsons 1972). Chemical parameters for gut contents were not all normally distributed, and we used nonparametric statistics when analyzing this portion of the data.

For stable isotopes, fish were collected from unbaited traps placed in L114 on 17 July 1994 and frozen. Guts were dissected and sampled as described above. A sample of 15 fish containing relatively high quantities of matter in both fore- and hindguts was chosen. For these,

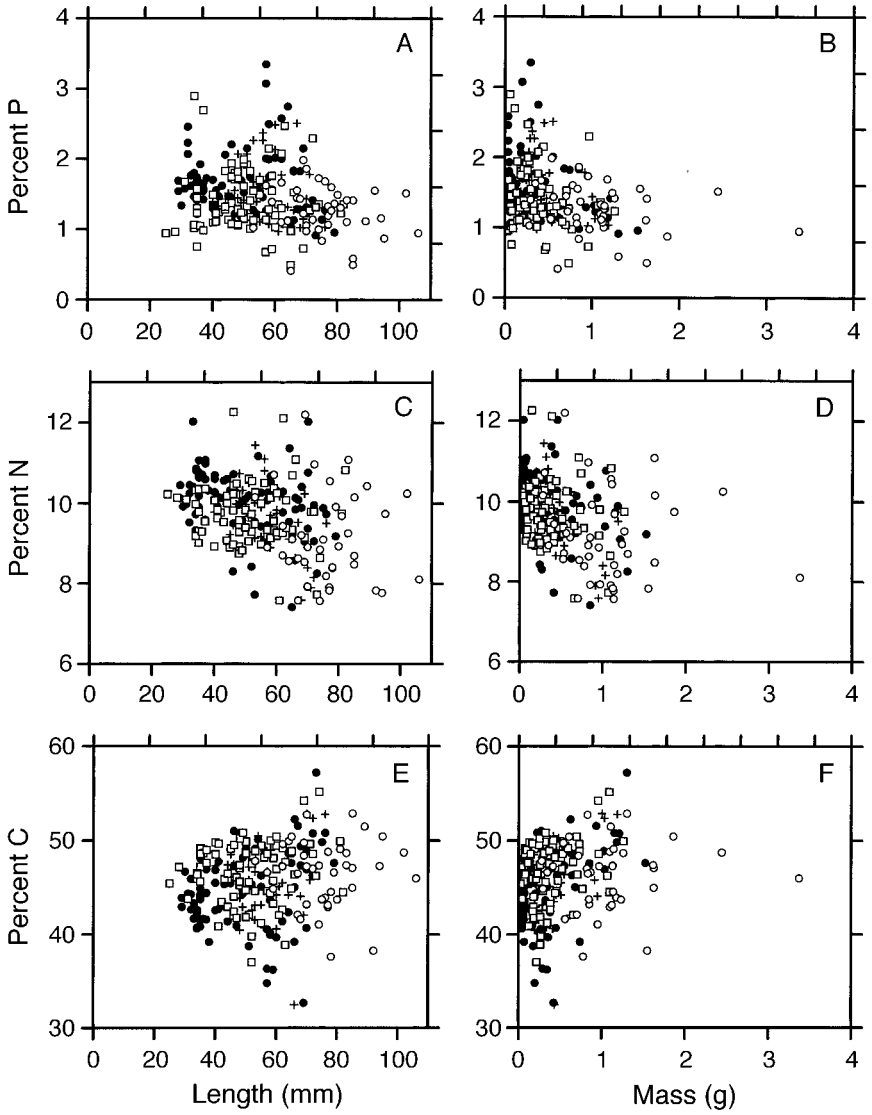

FIG. 2. Chemical composition of cyprinid minnows vs. fish size. Species are indicated as: ●, fathead minnows; □, northern red belly dace; +, finescale dace; ○, pearl dace. Correlations are given in Table 2.

the remaining fish ''body'' was freeze-dried and homogenized. Gut contents were freeze-dried, weighed (foregut mean, 3.4 mg; hindgut mean, 3.5 mg), and subsampled. Isotopes of C and N were determined at the Arizona State University Isotope Laboratory, Tempe, Arizona. Results are presented as δ values (‰) (Peterson and Fry 1987) relative to the customary standards (N: atmospheric N_2, C: PeeDee limestone). No replication was performed, but a single set of six duplicate body samples from a single fish were run to estimate precision ($\delta^{15}N$, SD = 0.10, 2% of mean; $\delta^{13}C$, SD = 1.15, 0.5% of mean).

RESULTS

Whole fish

Some weak but highly statistically significant allometric trends were observed (Figs. 2 and 3). Bivariate correlations were calculated between chemical variables and length and mass for individual species and the combined four-species sample (Table 2). Allometric patterns were most significant in fathead minnows. In this species, larger individuals were poorer in N and P than smaller individuals, but richer in C. Correlations were tighter with mass in this species, though all but one of the corresponding correlations with length were also significant. Trends were weaker or less consistent in the other three species, though all significant trends were in the same direction as in fathead minnows. No significant allometric relationships were observed in pearl dace. In the larger sample of cyprinids combined, all allometric correlations were statistically significant (Table 2). Longer or heavier fish in general were lower in P and N content relative either to body mass or to

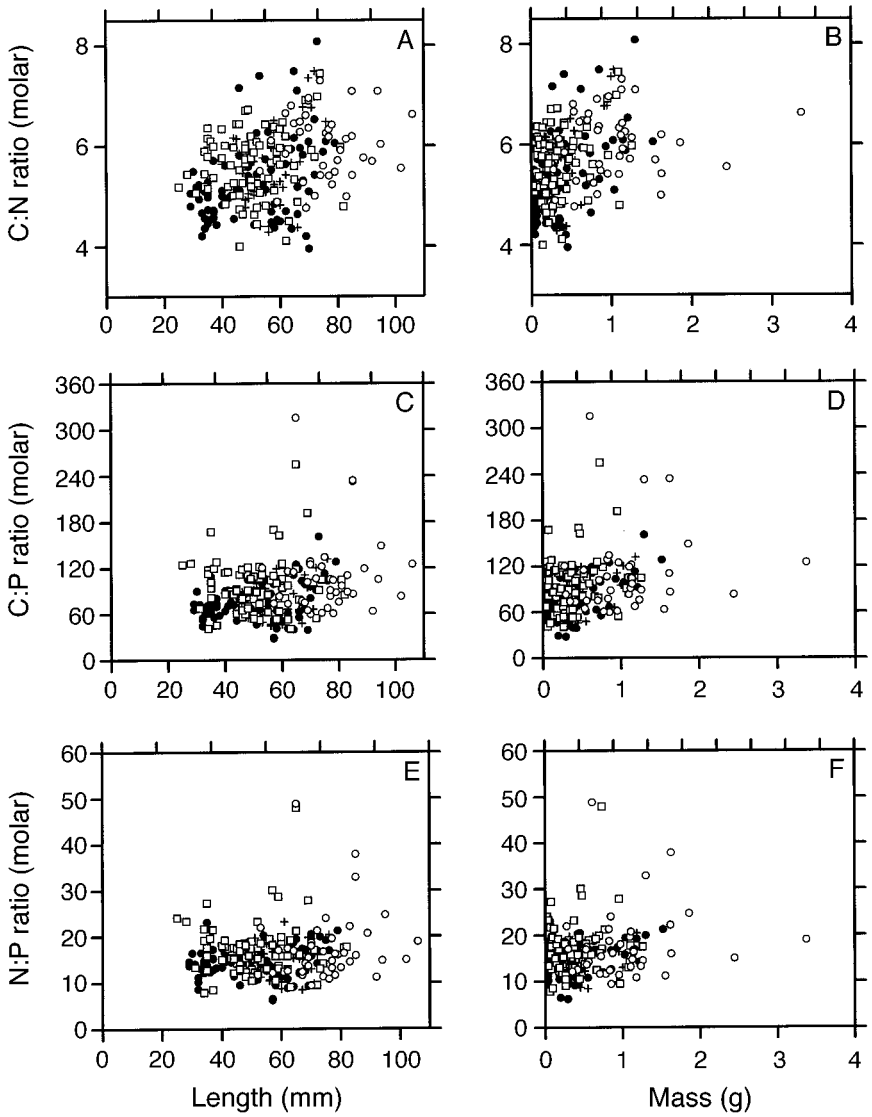

FIG. 3. Nutrient ratios of cyprinid minnows as a function of fish size. Species are indicated as in Fig. 2. Correlations are given in Table 2.

body carbon. Carbon as a percentage of dry mass was larger for larger fish. Thus, in general, larger cyprinids were less nutrient dense than smaller fish. The N:P ratio increased with both fish length or fish mass. Fish condition factor (a ratio of mass to length) was also significantly related to nutrient content (George 1994).

Formal tests of whether these allometric relationships were different within species but between lakes or months were not performed due to the limited sample sizes within some of these subsets of the data. Visual inspection of plots of the data did not indicate any strong evidence for heterogeneities of this kind. Tests for differences in mean values between lakes, between months, and their interaction, were then performed by two-way ANOVA, using length and mass as simultaneous covariates. The lake × month interaction was statistically significant in one of these 18 tests (three species times six chemical variables; PD lacked data from one lake by season combination so was not tested for interactions). The significant interaction was in percent carbon of northern red belly dace ($P = 0.02$). Of 48 possible main effects (four species times six chemical variables times two types of main effects, lake and season), six were statistically significant. Northern red belly dace had higher N:P ($P = 0.036$) and C:P ($P = 0.013$) ratios and lower percent P ($P = 0.016$) in L227 than L110. Fathead minnows had higher C:P ratios in later season samples than earlier in the season ($P = 0.007$). Finescale dace had higher C:N ratios ($P = 0.016$) and lower percent N ($P = 0.010$) in later season samples than earlier. Pearl dace showed no significant differences. In no cases did fish chemical content relate

TABLE 2. Bivariate correlations of nutrient ratios and fish size (length and mass).

		Length						Mass	
Species	*N*	%P	%N	%C	N:P	C:P	C:N	%P	%N
FH	83†	···	−0.341**	0.261*	···	0.305**	0.136***	−0.346**	−0.397***
FS	42	···	−0.456**	···	···	···	0.125*	···	−0.582***
NRBD	78	···	···	···	···	···	···	···	···
PD	39	···	···	···	···	···	···	···	···
Cyprinids	242‡	−0.193**	−0.347**	0.169**	0.144*	0.249***	0.351***	−0.309***	−0.379***

Notes: Only correlations significant at $P < 0.05$ are reported. Values given are correlation coefficients (r). Statistical significance is indicated by $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$. See Figs. 2 and 3 for plots of the data. Fish abbreviations are as in Table 1.

† Sample size = 82 for correlations with mass.

‡ Sample size = 241 for correlations with mass.

statistically to the identity of the lake. Given the rather large number of comparisons examined and the borderline significance of at least one of these specific comparisons, and given that differences between groups were small even when significant, the overall pattern is of constancy of fish nutrient content across lakes and across seasons, with some small and weak exceptions.

To test for interspecific differences in nutrient content of whole fish, fixed effect ANOVAs were performed on each chemical variable using fish length and fish mass as concurrent covariates. Differences among the species were small, but in some cases they were highly statistically significant. Concentrations of N and P generally declined in the order FH, FS, NRBD, PD, though not all of these differences were statistically significant (Fig. 4). Since differences among species were small, a grand average of all observations on all fish was also calculated (Table 3).

Fish gut contents

Extruded gut contents were mostly homogeneous and could not be identified. Table 3 presents the chemical characteristics, and frequency distributions are shown in Fig. 5. Most gut content masses were <1 mg. Median gut content masses were similar for fore- and hindguts and were ~0.4 mg. Some measurements on gut contents were clearly non-normally distributed both in terms of skewness (for symmetrical distributions, skewness = 0) and in terms of kurtosis (degree of ''peakedness,'' for normal distributions, kurtosis = 0). This statistical distribution for mass of gut contents reflects that fact that most fish have very small gut contents, but a few fish can be observed to have large mass in foregut, hindgut, or both. Chemical variables were closer to being normally distributed, though some of these variables also showed some skewing and lack of symmetry (Fig. 5). Gut content chemical variables that were close to being normally distributed were %N, %C, and the N:P ratio.

Measurements of gut masses and chemical variables were first examined for interspecific patterns. With the exception of foregut mass, which was noticeably larger (~5×) in PD than the other species (Kruskal-Wallis ANOVA, $P < 0.001$), none of the variables were significantly related to species, so data for all species were combined. Differences between elemental content of fore- and hindguts were small, but were significant for C (Mann-Whitney U test, $P = 0.01$) and N ($P = 0.001$). Hindguts had somewhat lower C and N content than foreguts. Mean P in foreguts was higher than hindguts, but the difference was not statistically significant ($P = 0.46$). None of the N:P ($P = 0.14$), the C:P ($P = 0.48$), or C:N ratios ($P = 0.14$) was statistically different between fore- and hindguts.

Though the chemical content of the fish themselves did not depend upon the lake they were caught in, there were some differences in their gut content chemistry across lakes. Specifically, fathead minnows from L110 showed higher C:N ratios (Fig. 6) and lower N content (not shown) in both foregut and hindgut than fish of the same species from other lakes. A somewhat less obvious, but still significant trend was also seen in northern red belly dace foreguts but not hindguts (Fig. 6). No other gut content chemical variable was different across lakes.

Spearman rank correlations were used first to discern linear association between the various components of gut content chemistry (Table 4). Cases of obvious autocorrelation (such as %P and C:P ratio, both of which are dependent upon the same measurement of P) are reported, but are not further interpreted. Note that chemical variables expressed as percentages and gut masses are not autocorrelated because they came from separate mass determinations. Patterns across the fish could be divided into two types: allometric and chemical. Not surprisingly, longer fish had larger fore- and hindgut masses, but they also had lower %P and higher C:P ratios in their foreguts. Correspondingly, larger foregut masses were lower in %P and hence had higher N:P and C:P ratios; similar trends although with weaker (sometimes nonsignificant) correlations were seen in comparing foregut mass to hindgut chemistry. Chemical variables showed few significant correlations. There was a fairly strong positive correlation between the %N in the fore- and hindguts as well as in the C:N ratios between fore- and hindguts. The rest of the correlations were not significant (Table 4).

TABLE 2. Extended.

Mass			
%C	N:P	C:P	C:N
0.497***	0.111**	0.543***	0.309***
...	...	0.171**	0.404***
0.302**	...	...	0.096**
...	...	...	...
0.287***	0.222***	0.343***	0.444***

Rank order correlations suggested a linear association between the N contents in foreguts and hindguts, but evaluation of the different scenarios in Fig. 1 requires more than correlation coefficients. Fortunately, both the %N and the C:N ratios were close to normally distributed, so linear regressions of pairs of these variables was performed (Fig. 7). Both slopes and intercepts were significantly different from zero. Slopes were less than one, though the scatter in the data was rather large.

Comparison of whole fish and fish gut contents

Although whole fish and fish gut contents came from samples from different years, the small amount of variability in the whole fish across lakes, seasons, and even species, led us to make a direct comparison between the whole fish data and the data for fish gut contents. We believe it is a safe assumption that the chemical

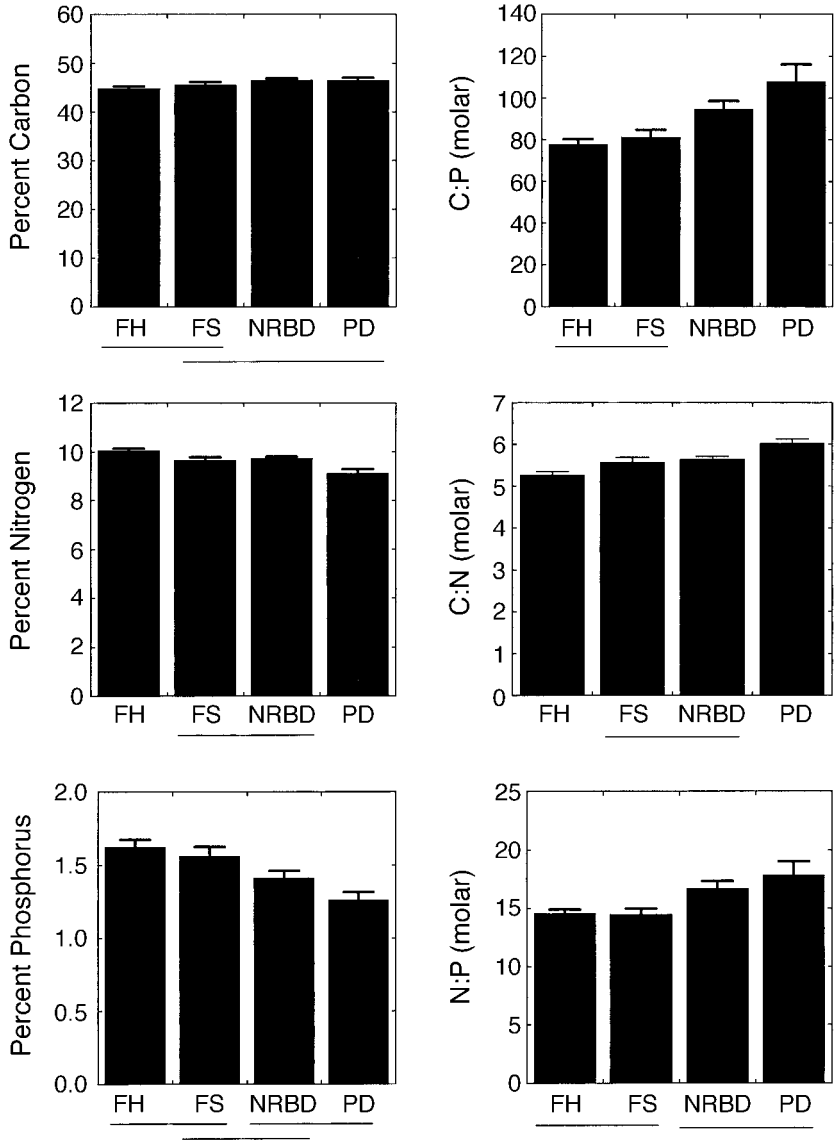

FIG. 4. Interspecific variation in cyprinid minnow nutrient chemistry (mean + 1 SE). ANOVAs using fish length and mass as covariates are all significant ($P < 0.02$). Homogeneous groups as determined by LSD are indicated by underlining. Abbreviations: NRBD, northern redbelly dace; FS, finescale dace; PD, pearl dace; FH, fathead minnows.

TABLE 3. Characteristics of the whole fish as well as the fore- and hindgut samples. See also Fig. 5.

Sample	*N*	Mean	Median	SD	Skewness	Kurtosis
Foregut, mass (mg)	133	1.05	0.41	2.4	6.11	43.8
Hindgut, mass (mg)	138	0.92	0.44	1.3	4.58	32.6
Foregut, C%	124	49.8	50.3	6.2	−2.09	10.0
Hindgut, C%	133	48.0	47.6	8.8	0.018	7.09
Whole fish, C%	242	45.7	46.0	3.8	−0.42	0.74
Foregut, N%	122	7.82	7.84	2.37	1.52	10.2
Hindgut, N%	127	7.30	7.06	2.1	1.95	12.5
Whole fish, N%	242	9.70	9.79	0.90	−0.14	0.36
Foregut, P%	138	1.58	1.13	1.77	4.11	20.2
Hindgut, P%	138	1.27	1.00	0.99	2.37	6.36
Whole fish, P%	242	1.49	1.41	0.45	0.92	1.76
Foregut, N:P	120	17.8	15.4	12.9	2.6	9.10
Hindgut, N:P	126	18.2	15.4	14.5	3.6	20.7
Whole fish, N:P	242	15.7	15.2	5.23	2.64	12.96
Foregut, C:P	122	135	115	100	2.8	10.9
Hindgut, C:P	128	135	118	85	1.94	6.4
Whole fish, C:P	242	88.5	83.7	35.2	2.28	10.1
Foregut C:N	118	7.76	7.45	2.05	1.1	3.4
Hindgut, C:N	122	8.04	7.77	1.97	0.72	1.45
Whole fish, C:N	242	5.55	5.51	0.76	0.42	−0.12

content of whole fish in 1993 would have been the same as in 1992. Also, gut content samples came from four lakes whereas whole fish came only from two of those four. There were few differences in gut chemistry across lakes, except for L110 compared to the other lakes. Since L110 is represented in both the whole fish and the gut content data, the comparison between these data sets seems to be valid. A more serious problem is an expected difference in analytical variability between the whole fish samples and the gut content samples. This difference would particularly be a problem where gut content mass was relatively small, since analytical error there might be considerably higher than for larger samples. We examined the entire gut content data as well as a smaller data set of gut contents with mass greater than the median mass. The smaller data set did not change our conclusions, and we present the full data here.

The N and P contents of the whole fish were higher than their gut contents (Fig. 5, Table 3), meaning these fish were more nutrient rich than what they eat. Very clear differences between the whole fish and their guts were seen in both percent P and percent N (Fig. 5A, B). A smaller difference and in the opposite direction was visible in percent C (Fig. 5C). Differences consistent with these trends were observed in the C:P and C:N ratios (Fig. 5D, E). The only chemical parameter where no difference between whole fish and their gut contents was apparent was the N:P ratio (Fig. 5F).

Fish were less variable chemically than their gut contents (Fig. 5). For all parameters, the standard deviation of foregut and hindgut contents was greater than for whole fish (Table 3). Chemical parameters were less variable in the smaller data set composed of gut masses greater than the median, but standard deviations still exceeded those for whole fish. Though the average N:P of minnows was similar to their gut contents, the variability was noticeably less. Thus, the fish appeared to regulate a homeostatic C, N, and P content.

Stable isotopes

Stable isotope analysis revealed differences between the isotopic signatures of the fish bodies and their gut contents (Fig. 8) (ANOVA, $P < 0.01$ for both C and N). For both N and C, fish bodies were isotopically heavy compared to their gut contents, whereas fore- and hindgut contents had similar isotopic composition (paired t tests, $P > 0.05$). Across fish, the fore- and hindgut contents were not correlated for $\delta^{15}N$ ($r = 0.38$, $P = 0.17$) but were correlated for $\delta^{13}C$ ($r = 0.55$, $P = 0.035$).

DISCUSSION

Studies of C:N:P stoichiometry in crustacean zooplankton have emphasized the wide interspecific variation in element composition relative to intraspecific variation (Sterner and Hessen 1994). The N and P contents of zooplankton are also more constrained than their algal food. Cyprinids exhibit similar patterns. The N and P contents of the fish were more constrained than their resources; thus, the fish must maintain an active control of their nutrient content. For example, fathead minnows in L110 consumed food with C:N ratio of about 10.5 but fathead minnows in L114 and L227 consumed food with C:N ratio of about 7; nevertheless, the C:N ratio of the minnows was the same in these lakes. To maintain the same C:N ratio in fish tissue in the different lakes, the minnows in L110 must have a lower gross growth efficiency in terms of C or a higher efficiency in terms of N than do the fish in the other lakes.

To date there has been no comparative study of N and P content in fish across major groups. We compared our whole-fish results to other studies (Fig. 9). Across

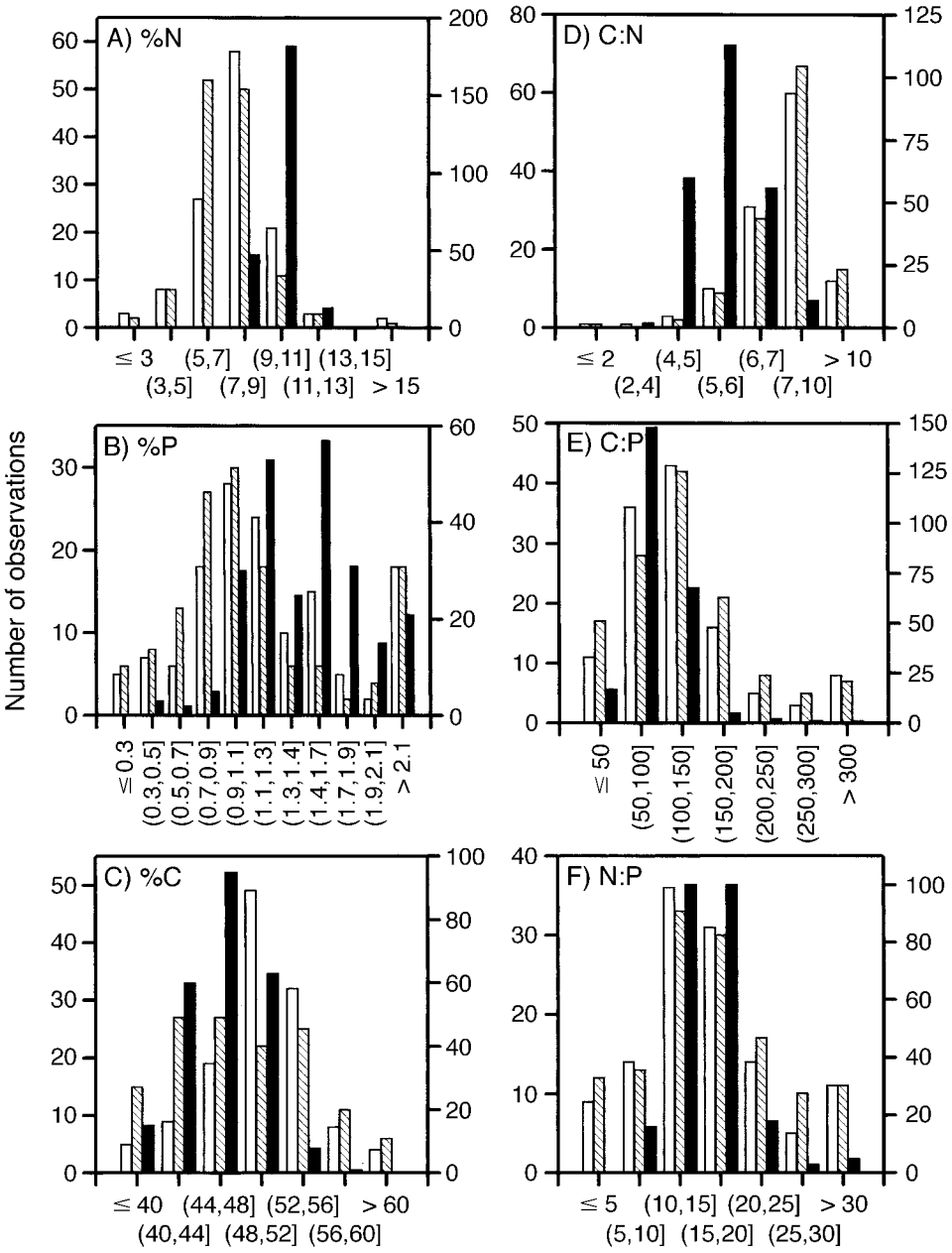

FIG 5. Frequency distributions of chemical variables in whole fish, foreguts, and hindguts: open bars, foregut; shaded bars, hindgut; solid bars, whole fish. Numbers given are total observations within the designated interval. In each panel, the left vertical axis scale pertains to the number of gut samples, while the right vertical axis scale pertains to the number of whole fish observations. See also Table 3.

species, fish N content varies little, with a range from ~8% to 11% and a mean close to 10%. In contrast, fish P content varies from ~1% to 5%. The major taxonomic pattern was that members of two closely related families, Percidae and Centrarchidae, had high P content. Nakashima and Leggett (1980*a*) also observed a high P content (3.1%) in *Perca* compared to two other species from other families (*Osmerus,* 1.6% and *Notemigonus,* 2.5%; they did not present N content, so these data are not plotted in Fig. 9). We speculate that P content in fish relates to their ''boniness'' and that the soft-bodied cyprinids in our study have less skeletal development than most other fish so far studied. Fish N:P ratios vary from about five to about 15, with the species of high P content at the lower end of that scale. We note that the wide variation in P content in fish is similar to freshwater zooplankton (Sterner and Hessen 1994), though the P content in fish is considerably high-

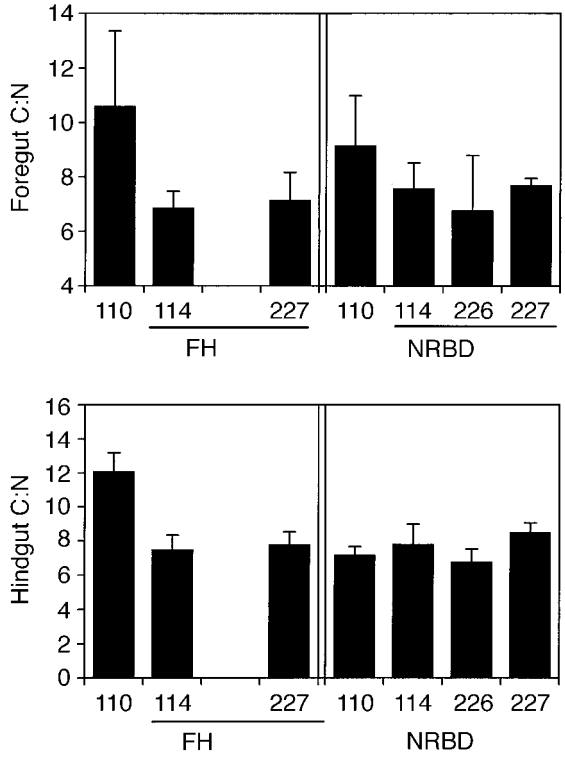

FIG. 6. Carbon : nitrogen ratios of gut contents in fathead minnows (FH) and northern red belly dace (NRBD) in four study lakes (110, 114, 226, 227). Bars indicate medians, and error bars represent the 75% quartiles. ANOVA showed statistical significance for all except hindgut C:N in NRBD. Horizontal lines indicate homogeneous groups as determined by LSD tests. There are no observations of gut content chemical content for fathead minnows in lake 226.

er than in zooplankton where the range is from ~0.5% to ~1.5%. In zooplankton, it is hypothesized that P content relates to life history evolution and a coupling between RNA and growth rate (Elser et al. 1996, Main et al. 1997). It is an open question whether there are similar couplings in fish.

The nutrient allometry of these cyprinids differed from the centrarchid *Lepomis macrochirus* (Davis and Boyd 1978). Bluegills in that study had lower N content with increasing size, similar to the trends we observed for cyprinids. However, bluegill P content increased with fish length, while in the cyprinids, we observed P content to decline with fish size (observed in FH as well as all cyprinids). In bluegills, the N:P ratio declined with fish size, while in the cyprinids, the N:P ratio increased with fish size.

Stoichiometric models of nutrient recycling have been based on enhanced constraint of nutrient content in consumers compared to their resources (Sterner 1990). A model combining nutrient stoichiometry with energetics was recently described for fish (Schindler and Eby 1997). Application of this model indicated that fish in nature were energy rather than nutrient limited, and as a consequence fish generally recycled nutrients in similar N:P ratios as ingested. In the four species of minnows studied here, the N:P ratio ingested was nearly identical to the N:P ratio of the fish themselves, another factor (which would cause the N:P recycled to be similar to the N:P ratio ingested. We can deduce that the cyprinids in this study egest and excrete nutrients at an N:P ratio close to 15. This N:P ratio is similar to other values measured or modeled in fish nutrient release (Schaus et al. 1997, Schindler and Eby 1997).

If assimilation efficiencies are not adjusted to maintain homeostasis, a pattern such as in Fig. 1A is ex-

TABLE 4. Spearman rank-order correlation matrix for full data set (all fish).

	Foregut							Hindgut	
Length	Mass	%C	%N	%P	N:P	C:P	C:N	Mass	%C
Fish length	0.442*	0.148	0.054	−0.184*	0.172	0.240*	0.078	0.372*	0.123
Foregut									
Mass	···	−0.046	0.051	−0.312*	0.267*	0.271*	0.004	0.361*	0.047
%C		···	0.119	−0.246*	0.237*	(0.450)	(0.313)	−0.248*	0.379*
%N			···	0.123	(0.431)	−0.045	(−0.839)	−0.105	0.128
%P				···	(−0.754)	(−0.993)	−0.211*	−0.107	0.031
N:P					···	0.781	(−0.224)	0.076	−0.047
C:P						···	(0.266)	0.033	0.036
C:N							···	0.053	0.007
Hindgut									
Mass								···	−0.094
%C									···
%N									
%P									
N:P									
C:P									

Notes: Table entries are correlation coefficients (n = 107–115). Correlation coefficients for autocorrelated variables are reported in parentheses; statistical significance is not indicated for these coefficients.

* $P < 0.05$.

pected. Most bioenergetic modeling assumes such a constant assimilation efficiency of digestion of individual elements, but our data do not support such a passive model. The passive model is inconsistent with the observed homeostasis in C:N:P in the whole fish. Also, regressions of hindgut N content (as percentage or as ratio with C) vs. foregut N content demonstrated significant slopes and intercepts ($P < 0.002$) (Fig. 7). The nonzero *y*-intercepts are statistical evidence of adjustment of absorption as a function of nutrient content of ingested food. However, the trends of these adjustments are somewhat surprising. Though the data are quite variable, particularly for C:N ratios (Fig. 7), our data conform best to the pattern indicated in Fig. 1B, line 2. Slopes <1 in Fig. 7 imply that fish with a high-N diet assimilate N more efficiently than those with an N-poor diet, reverse of what can maintain nutrient homeostasis. If this is true, maintenance of fish homeostasis must be occurring at the level of physiological processing of already assimilated nutrients.

Stable isotopes revealed a number of things about C and N in these fish. Hecky and Hesslein (1995) reported an extensive set of measurements of C and N isotopes in various members of the biota of ELA lakes. In general, not all lakes have the same baseline $\delta^{15}N$ in their algae. However, $\delta^{15}N$ baselines in ELA lakes are fairly consistent across lakes and seasons (Hecky and Hesslein 1995). Therefore, Hecky and Hesslein considered plants (not sampled) to be trophic level 1, and they then defined trophic level 2 as animals with $\delta^{15}N <$ 6‰, level 3 as 6–9‰, and level 4 as >9‰. They do not report values for the four species of fish examined in this paper; most of their fish were larger species. Fish in their study were found primarily to be occupying trophic levels 3 and 4, though a minority of individuals occupied trophic level 2. The cyprinids in the present study had N isotopic signatures near the bottom of the range of the fish examined by Hecky and Hesslein, indicating that the cyprinids probably occupied a somewhat lower trophic feeding position and would be at their upper end of the range for trophic level 2.

Fractionation at the air–water interface means that phytoplankton should have C isotopic signatures lighter than these terrestrial values; it is hypothesized to be about −36 (Hecky and Hesslein 1995). Diffusion

TABLE 4. Extended.

Hindgut				
%N	%P	N:P	C:P	C:N
0.089	0.042	-0.017	0.035	0.065
0.077	−0.114	0.141	0.183	0.105
0.136	0.100	−0.096	−0.032	0.069
0.475*	0.042	0.060	−0.055	−0.330*
0.050	0.023	−0.003	−0.069	−0.111
0.148	−0.082	0.102	0.055	−0.086
−0.109	0.062	0.143	0.113	0.117
−0.300*	0.025	−0.103	−0.006	0.263*
−0.090	−0.300*	0.191*	0.247*	0.172
0.229*	−0.141	0.169	(0.338)	(0.374)
···	−0.042	(0.384)	0.081	(−0.779)
	···	(−0.890)	(−0.962)	−0.139
		···	(0.892)	(−0.178)
			···	(0.196)

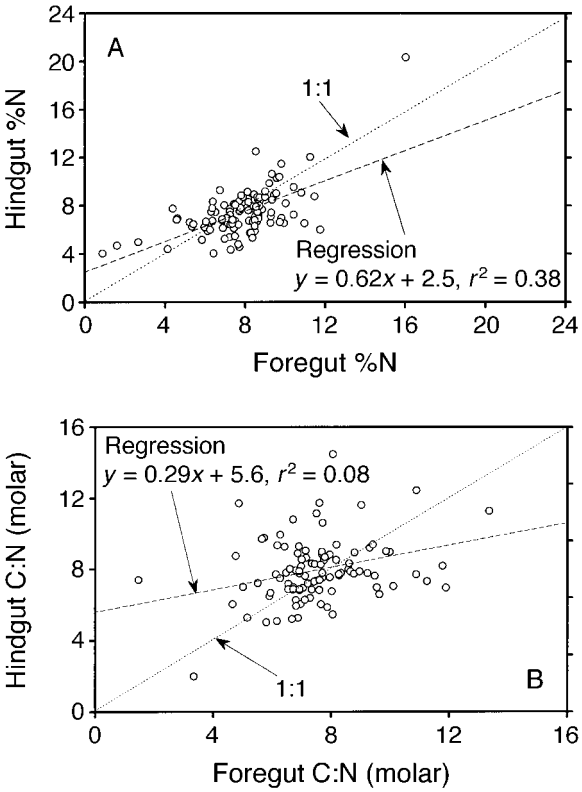

FIG. 7. Comparison of N content in fore- and hindguts. P content was not correlated between fore- and hindguts.

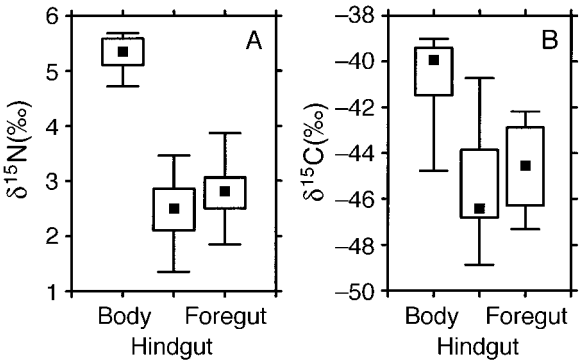

FIG. 8. Stable isotope signatures of body, foregut, and hindgut of cyprinid minnows for (A) nitrogen and (B) carbon. Solid squares denote median values; rectangles enclose the middle two quartiles; and whiskers show range ($n = 15$ fish).

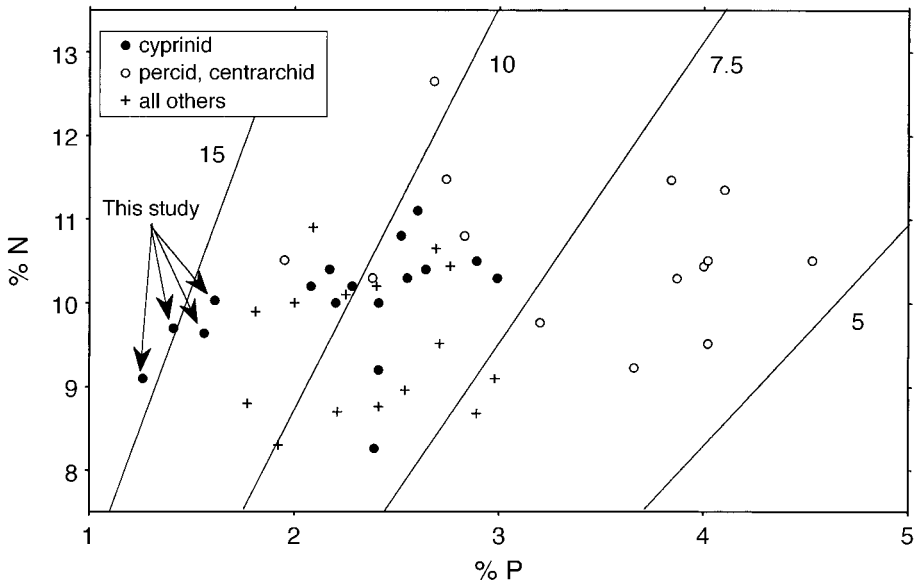

FIG. 9. Plot of N and P content of various freshwater fishes. The correlation between %N and %P is not significant. Solid lines indicate the labeled molar N:P ratios. Data are from this study, Davis and Boyd (1978: Table 3), Goodyear and Boyd (1972: Table 2), Penczak (1985: Table 1), and Penczak et al. (1985: Table 1).

boundary layers come into play in benthic photosynthesis, and for these autotrophs, C isotopic signatures over a wide range have been measured, but all are heavier than phytoplankton. The C signatures of the cyprinids in this study were isotopically light, closer to expectations for phytoplankton than for terrestrial C or benthic algae. In fact, the minnows were even somewhat lighter than expected for phytoplankton. These data indicate that the majority of minnow C comes from planktonic production rather than from terrestrial or benthic sources.

An increase of $\delta^{15}N$ between fish gut contents and fish bodies of ~3.4‰ is expected from previous studies, showing this magnitude of difference across single tropic levels (Minagawa and Wada 1984, Peterson and Fry 1987, Vander Zanden et al. 1997). Minnows in this study showed near perfect agreement with the 3.4‰ expectation (Fig. 8A). N sampled in the guts must not have been strongly influenced by N excretion into the digestive tract, for example in the form of digestive enzymes, since these should have an N signal closer to the fish bodies than to the fish foreguts. No large consistent difference in $\delta^{13}C$ between resources and consumers is expected (DeNiro and Epstein 1978, Peterson and Fry 1987, Hesslein et al. 1991), but some difference was in any case observed (Fig. 8B). In contrast to the relative constancy of $\delta^{15}N$, there is a considerable seasonal change in $\delta^{13}C$ in ELA lakes (R. Hesslein, *personal communication*). Cyprinid bodies would average over a long time period relative to the gut contents. Such a seasonal shift could lie behind the differences observed in Fig. 8B.

The major features of cyprinid C:N:P stoichiometry that this study has revealed are: (1) Minnow nutrient content shows minor variation with fish size and season; (2) Minnow nutrient content is constant across lakes, even if the chemical composition of their food is not; (3) Minnows consume resources that are lower in P and N but higher in C than are the minnows themselves; (4) The N:P ratio of minnows is generally similar to the N:P ratio of what they consume; (5) Maintenance of homeostasis is largely a result of differential processing of assimilated nutrients rather than adjustments to digestive activities; and (6) Minnows show a 3‰ increase in ^{15}N compared to their resources. In addition, we have described, we think for the first time, some of the taxonomic correlates for fish nutrient content. These results contribute to the ongoing clarification of the role of fish in nutrient cycling in lakes. They also help generalize the stoichiometric models developed for zooplankton and their resources.

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