Journal of Fish Biology (2007) **70,** 121–140
doi:10.1111/j.1095-8649.2006.01280.x, available online at http://www.blackwell-synergy.com

Elemental stoichiometry of freshwater fishes in relation to phylogeny, allometry and ecology

H. A. Hendrixson*, R. W. Sterner† and A. D. Kay‡

Department of Ecology, Evolution and Behavior, University of Minnesota, St Paul, MN 55108, U.S.A.

(Received 28 November 2005, Accepted 3 August 2006)

Twenty species of freshwater fishes were collected from Minnesota, Iowa and Michigan and their whole-body carbon, nitrogen and phosphorus contents and the respective C:N:P ratios were determined. Patterns were examined in intra- and interspecific variation, allometry and variation caused by habitat and trophic level in whole fish while controlling for the role of phylogeny. Stoichiometric variation was greater across than within species, C:N:P allometry was species-specific, nutrient content within a species was somewhat habitat-specific and P concentration showed a strong phylogenetic signal. Stoichiometric relationships with allometry and feeding guild were observed but were not significant in an analysis accounting for non-independence of closely related species. Supportive evidence for the hypothesis that the considerable variation in whole fish phosphorus concentrations could be ascribed to differences in bone and scale development, as previously suggested, is shown. Whole fish Ca:P ratios had a nearly constant stoichiometry consistent with the chemical signature of bone. This result combined with a phylogenetic signal for fish P indicated that the great stoichiometric variability among fish taxa in P content was derived almost entirely from skeletal investment. © 2007 The Authors
Journal compilation © 2007 The Fisheries Society of the British Isles

Key words: allometry; calcium; ecological stoichiometry; phosphorus; phylogeny.

INTRODUCTION

Elemental composition, or the stoichiometry of individual organisms, is a taxon-dependent trait, ultimately arising from evolutionary pressures on form and function (Williams & Fraústo da Silva, 1996; Sterner & Elser, 2002). To understand how selective regimes shape stoichiometry, the relationship between elemental composition, phenotypes and functional performance must be established (Kay *et al.*, 2005). Two major evolutionary factors that have been hypothesized to influence the stoichiometry of animals are: 1) the growth rate hypothesis, which concerns positive correlations among P content, growth rate and RNA concentration (Elser *et al.*, 2003) and 2) in vertebrates, structural

†Author to whom correspondence should be addressed. Tel.: +1 612 625 6790; fax: +1 612 624 6777; email: stern007@umn.edu
*Present address: The Nature Conservancy, 226 Pine Street, Klamath Falls, OR 97601, U.S.A.
‡Present address: Department of Biology, University of St Thomas, St Paul, MN 5510, U.S.A.

© 2007 The Authors
Journal compilation © 2007 The Fisheries Society of the British Isles

demands arise from skeletal investment (Sterner & Elser, 2002). The allometry of skeletal investment has been described for mammals (Prange *et al.*, 1979) and fishes (Cassadevall *et al.*, 1990). Elser *et al.* (1996) combined these two hypotheses and surmised that animals of intermediate size would be lowest in P content, because the smallest 'animals' (*e.g.* unicells and small metazoans) need high quantities of P-rich RNA to generate their high specific growth rates, and large vertebrates have high structural demand and high proportions of their body mass composed of bone. Gillooly *et al.* (2005) confirmed that RNA content does generally decline with 'body' size in samples composed of uni-cellular organisms and multicellular animals (14 orders of magnitude range in size), as well as within more taxon-specific samples including 'birds and mammals' and 'marine and freshwater fish'.

Studies of the C:N:P stoichiometry of freshwater fishes have so far concerned a limited number of species or families (Goodyear & Boyd, 1972; Davis & Boyd, 1978; Sterner & George, 2000; Vanni *et al.*, 2002), or several species in a limited habitat range (Penczak, 1985; Tanner *et al.*, 2000). Previous studies have noted that high-P fishes are typically bony or protected by hard integument, and it has thus been suggested that the 'boniness' is a factor in determining whole-body P concentration in fishes due to bone's high-P content. To date, however, there has been little study of wild fish C:N:P content with broad taxonomic coverage or across a wide geographic range. Hence, the variability and patterns in fish nutrient content have not been fully explored, and warrant more examination.

In addition to the internal skeleton present in all vertebrates, fishes also possess calcified scales (Moyle & Cech, 2004) that can contribute to organismal stoichiometry. The two main types of teleost scales, ctenoid and cycloid, differ only slightly in form and function. Ctenoid scales have small projections on their outer edge making the fishes feel rough; they are generally found in spiny-rayed fishes. In contrast, cycloid scales are smooth and are generally found in fishes that lack true spines such as salmonids and cyprinids. Skeletal and scale ossification are reduced at high growth rate (Arendt *et al.*, 2001).

The total variation in elemental content observed among individuals within a single species can arise from several sources: 1) sex, stage, size or other similar life-history characteristic, 2) habitat (excluding food) and 3) feeding history. Field-collected fishes will reflect variability from all these sources. Stoichiometric homoeostasis, defined as a 'narrowing of variation in chemical content in an organism compared to the resources it consumes' (Sterner & Elser, 2002), dampens the influence of feeding history on fish nutrient composition. Stoichiometric homeostasis relates to feeding history after accounting for the other two factors named above. In one study, cyprinids were found to show small variation in % N and % P (9–10% N, 1·25–1·6% P) across several lakes of differing productivity (Sterner & George, 2000). Similarly, small differences in elemental concentrations of largemouth bass *Micropterus salmoides* (Lacepède) (3·6–4·27% P, 11·16–11·90% N) were observed in five sites differing in dissolved nutrient concentrations (Ca, Mg, K and Na) (Goodyear & Boyd, 1972). Patterns in nutrient content across species add additional, potentially phylogenetically based, variation to organismal stoichiometric patterns. Concentrations of nutrients, especially P, differ substantially among fish species.

© 2007 The Authors

For example, 26 species of tropical stream fishes in Venezuela were found to vary little in C or N but greatly in P (Vanni *et al.*, 2002). Stoichiometric variation was seen to correspond mainly to high taxonomic organizational levels, family or above.

Many biological features scale to body size, including metabolism, temperature and production (Peters, 1983). Nutrient concentration may also scale allometrically. Almost all studies of fish stoichiometry to date have included an attempt to relate nutrient concentration to size or age. Allometric patterns have indeed been identified, but have not been consistent in magnitude or direction across studies. For example, in bluegill *Lepomis macrochirus* (Rafinesque) the N:P ratio decreased with size (Davis & Boyd, 1978), but in cyprinid minnows N:P increased with size (Sterner & George, 2000). Most studies of the allometry of fish stoichiometry predate recent statistical and conceptual developments in comparative biology, which take into account non-independence of closely related species.

'Phylogenetically independent contrasts' (Felsenstein, 1985) are an increasingly accepted way to utilize comparative data. Phylogenetically independent contrasts take into account the phylogenetic relatedness among species in a comparative study. They address the potential problem of inflating statistical significance that could be seen if species relatedness is not taken into account because each species cannot be considered an independent statistical event. Phylogenetically informed analyses are still relatively new in ecological stoichiometry (Kay *et al.*, 2005) but have been used in two studies on insects (Fagan *et al.*, 2002; Woods *et al.*, 2004).

Fish biomass may constitute a large ecosystem-scale pool of nutrients such as P (Kitchell *et al.*, 1975; Nakashima & Leggett, 1980; Kraft, 1992). Fishes can be as important as zooplankton in recycling N and P in open water systems (Tatrai, 1987; Vanni & Findlay, 1990; Schindler & Eby, 1997; Schaus *et al.*, 1997). They may also be important in streams (Vanni *et al.*, 2002; Hood *et al.*, 2005) and in systems where fishes feed primarily on the benthos (Hecky & Hesslein, 1995). Benthic-feeding fishes can transport nutrients to the pelagic zones of lakes from sediments and provide a new source for algae (Brabrand *et al.*, 1990). As external P loading from a watershed decreases, fish feeding on sediments and subsequently releasing P have been shown to become an important source of P to the epilimnion of lakes (Brabrand *et al.*, 1990). Nutrient content has been used to assess the extent to which fishes affect nutrient cycling and recycling rates (Kitchell *et al.*, 1975; Nakashima & Leggett, 1980; Kraft, 1992; Schindler & Eby, 1997; Vanni *et al.*, 1997, 2002; Attayde & Hansson, 2000). An imbalance between the nutrient ratio of a fish's body and its food will affect the rate and ratios of recycled nutrients (Vanni *et al.*, 2002).

Elements from dead fishes may also enter ecosystem nutrient cycles. Decomposing fishes release nutrients that may have ecosystem-level impact (Threlkeld, 1988). Fish stoichiometry has recently been used to examine the impact of fish decomposition and removal through harvesting on nutrient dynamics. Decomposing rainbow trout *Oncorhynchus mykiss* (Walbaum) carcasses have been shown to lose 95% of the original N and 60% of the original P into the water over a 10 month period (Parmenter & Lamarra, 1991). Removal of fishes *via* capture has been examined as a way to reduce nutrients in the Baltic Sea

© 2007 The Authors

(Hjerne & Hansson, 2002) and in hyper-eutrophic Lake Donghu in China (Tang & Xie, 2000), where fish catch removes *c.* 10% of the external P load annually.

Body N:P ratios are critical in understanding nutrient cycling because body N:P helps determine the relative efficiencies with which these two potentially limiting nutrients will be recycled (Sterner, 1990; Kraft, 1992; Elser *et al.*, 1996). Tanner *et al.* (2000) pointed out that mean values for C, N and P in fishes can be used to construct nutrient budgets, but where interspecific variation is large and fish community structure varies, it is crucial to have specific nutrient values for individual species. This type of data will give insight into how trophic levels (*i.e.* producers, primary consumers and secondary consumers) relate to one another, and how different species affect and are affected by the nutrients around them.

The central goals of this study were: 1) to assess the overall variation in fish stoichiometry in a large sampling of species, 2) to parse that variation out among sources, including within *v.* among species, size and habitat and 3) to examine the phylogenetic patterns in fish stoichiometry.

MATERIALS AND METHODS

COLLECTING

Fishes were collected by seining or angling in 33 lakes in four geographic regions within Minnesota, Iowa and Michigan during the summers of 2000 and 2001. A range of sizes of individuals of 20 species were collected in single lakes, and several common species were sampled from multiple lakes. Lakes were chosen to vary widely in productivity, depth and area. Lakes ranged from tens to hundreds of hectares in surface area. Lakes in northern Iowa and southern Minnesota were in the vicinity of the Iowa Lakeside Laboratory. Lakes in the north-central region of Minnesota were located near Itasca State Park. Samples taken in northern Minnesota were close to the Boundary Waters Canoe Area, where the generally soft water, oligotrophic lakes lie in forested low-nutrient glacial till, which is thinner and less productive than the soils to the south. The fourth sampling was central Michigan near the Kellogg Biological Station, where the lakes tended to be deep and have hard water. Each lake was visited only once. After identifying the fishes, several of each size of a species were killed and promptly frozen. Additional species were obtained from the Minnesota Department of Natural Resources (MNDNR).

SAMPLE PREPARATION AND NUTRIENT DETERMINATION

Frozen fishes were defrosted in warm water and measured to the nearest mm for total length (L_T; tip of the snout to the end of the relaxed caudal fin). Gut contents were removed and the specimens were cut into small pieces to facilitate drying. The wet mass was measured to the nearest 0·1 mg, and specimens were dried at 60° C for at least 1 week, or until a constant mass was obtained, before grinding them in a Wiley mill. The Wiley mill had a 20 mesh screen and any material that did not pass through the mesh was cut into fine pieces with scissors and reground. The ground whole fish was then stored in a desiccator until analysis.

Duplicate samples from each fish were analysed for C and N using a Perkin-Elmer CHN-2400 Elemental Analyzer. Before measuring *c.* 1·5–2·5 mg of material into a tin capsule, the sample was re-dried at 60° C overnight and cooled in a desiccator to remove any moisture that might have accumulated before weighing.

© 2007 The Authors

Each individual was analysed for P, either with inductively coupled plasma atomic emission spectroscopy (ICP-AES, described below) or with the ammonium molybdate method after digestion with H_2SO_4. For the ammonium molybdate method, samples were ashed at 550° C for 8 h before digestion in hot 10 N H_2SO_4. Spinach (NIST 1570a) was used as a standard and P recoverability also was routinely determined with bovine reference material (NIST 8414). Approximately 60 mg of ground fish was used in both methods to reduce variability in results. Samples <60 mg yielded variable results. P samples processed using ICP-AES were comparable to those done using the ammonium molybdate method when samples >60 mg were used. The maximum difference in % P measured by the two methods was 0·36%.

The contribution of P to structural components (skeleton and scales) in different species of fishes was examined to determine how that investment can affect C:N:P within and across species. A conceptually straightforward way to do this would be to directly determine the amount of P in bone and multiply it by the skeletal mass for each fish. Unfortunately, there is limited information on skeletal mass of fishes (Cassadevall *et al.*, 1990) and this is a difficult measurement to make on large numbers of samples due to problems separating bone from other tissues. Therefore, a less direct method was used to address the role of skeleton in fish stoichiometry by analysing both Ca and P and examining their interrelationships.

ICP-AES was used to determine the concentrations of 15 different elements in the fish samples (Hendrixson, 2002). Here, only the data for P and Ca are presented. Eighty samples of whole fish from 18 of 20 species, and 11 skeletons, were chosen for analysis. All ICP-AES analyses were conducted by the Research Analytical Laboratory (RAL) at the University of Minnesota, using an Applied Research Laboratory 3560 ICP Spectrometer. To prepare samples, *c.* 60 mg of ground fish tissue, re-dried for 12 h before weighing, was placed in glass test tubes and ashed for 8 h at 550° C. Halfway through ashing, 10–20 µl concentrated nitric acid was added to facilitate removal of carbon. After ashing, samples were digested in 10% trace metal clean HCL and brought to RAL for analysis. Empty test tube blanks received the same treatment as samples and were used to check levels of background noise in the HCl matrix.

To obtain skeletons, 11 whole fishes from eight species were boiled in water for 45 min and the skeletons removed by hand. Skeletons were dried in a drying oven at 60° C for 48 h and then ground in a Wiley mill before being analysed using ICP-AES, as described above. The Ca:P ratio in bone samples was compared with the Ca:P in whole fishes to compute the amount of P in bone, then the P in whole fishes minus their skeletons was calculated.

STATISTICAL ANALYSES

One-way ANOVA was used to compare differences in untransformed C, N, P, C:N, C:P and N:P across species, unadjusted for L_T. *Post hoc* multiple comparisons were made using Tukey's HSD test.

Statistical treatment of data in comparative biology has undergone much revision as the need to incorporate knowledge of phylogeny is increasingly appreciated. Inaccurate calculation of type-1 error may result from a statistical test based purely on correlations observed among groups of closely related species, because each species cannot be treated as statistically independent. Blomberg *et al.* (2003) thus propose that a first step in analysis should be to ascertain the degree to which a given trait or traits relate to phylogeny, and they used the term 'phylogenetic signal' and devised the statistic K to describe that correspondence. Phylogenetic signal refers to the tendency for closely related species to resemble each other. Specifically, K compares the fit between the data and a given tree (with specified topology and branch lengths) to the fit obtained when the data are randomly assigned across the terminal nodes of the tree. $K = 1$ indicates that trait variation across species coincides with the tree structure (strong phylogenetic signal or 'phylogenetic inertia'); $K << 1$ indicates that related species resemble each other less than expected given the tree structure.

© 2007 The Authors

A composite tree was constructed using the computer programme MacClade, version 3.0. The phylogeny used (Hendrixson, 2002) was based on previously published relationships (Lundberg, 1970; Fink & Fink, 1981; Lauder & Liem, 1983; Siebert, 1987; Cavender & Coburn, 1991; Coburn & Cavender, 1991; Johnson & Patterson, 1996). All branches between nodes were assumed to be of equal length given a lack of more detailed information. The computer package PHYSIG was used (Blomberg *et al.*, 2003) to determine the extent to which whole fish C, N, and P concentrations were correlated with phylogeny (the phylogenetic signal). Within PHYSIG, a randomization test was conducted (with 1000 permutations of the tree structure) to determine the probability of obtaining a specific congruence between the data and the tree structure by chance. Because whole fish P concentration showed a strong phylogenetic signal, an independent contrasts analysis was used (Felsenstein, 1985) to evaluate the interspecific allometry of P and N:P ratios, and the interspecific relationships between C, N and P concentrations. Independent contrasts were determined using the PDAP programme (Garland *et al.*, 1992).

Simple linear regression analysis was used to look for allometric trends in nutrients, grouped by species. Relationships were examined based on linear regressions using L_T and power functions using mass, the latter for consistency with other allometric literature. To examine the effect of habitat on P in bluegill, the residuals from the linear regression of P on L_T across lakes was plotted and analysed with one-way ANOVA.

RESULTS

INTERSPECIFIC STOICHIOMETRIC PATTERNS

A total of 157 individuals from 20 species (Table I) were analysed for whole-body nutrient content. Specimens ranged from 33 to 477 mm L_T and 0·05–284 g dry mass. Considerable stoichiometric variability was found among species, particularly in phosphorus content. Fish P varied about two-fold, ranging from <2 to >4% of body mass [Fig. 1(a); $F_{19,137}$, $P < 0{\cdot}001$]. Fish N ranged from *c.* 9 to 12% [Fig. 1(b); $F_{19,137}$, $P < 0{\cdot}001$] and fish C was proportionately least variable, ranging from <40 to >50% ($F_{19,137}$, $P < 0{\cdot}001$). Species of low body P (left side, Fig. 1) were mostly members of the families Salmonidae, Cyprinidae and Catostomidae. High-P species (right side, Fig. 1) were mostly members of the families Centrarchidae, Esocidae and Ictaluridae. Across all species, there was a significant phylogenetic signal for whole fish P concentration ($K = 0{\cdot}789$, $P < 0{\cdot}002$). In contrast, C and N concentrations showed little phylogenetic signal (carbon: $K = 0{\cdot}476$, $P > 0{\cdot}05$; nitrogen: $K = 0{\cdot}315$, $P > 0{\cdot}05$).

Fish P and C content were stoichiometrically linked, with higher % P associated with lower % C (Fig. 2). The negative correlation between P and C content was also statistically significant in the phylogenetically corrected analysis ($r = 0{\cdot}82$, $P < 0{\cdot}001$). Type I regression was used to estimate the slope in this plot, even though both the x and y variables were measured with error. The stoichiometric linkage between C and P is not a simple consequence of one element 'displacing' the other within total body mass:carbon mass decreased almost ×4 with every mass unit increase in P; thus, some other elements must also make up a larger proportion of total biomass in high-P fishes. If type II regression had been used, the slope would be even larger, further emphasizing this point. The other element associated with P was not nitrogen: N content

© 2007 The Authors
Journal compilation © 2007 The Fisheries Society of the British Isles,

© 2007 The Authors
Journal compilation © 2007 The Fisheries Society of the British Isles, *Journal of Fish Biology* 2007, **70**, 121–140

TABLE I. Taxonomy, feeding type, samples size, sampling locations and size range (total length and mass) of fish species studied

Order	Family	Genus and species	Common name	Feeding type	Scale type	Sample size	Sampling locations	Range	
								L_T (mm)	Dry mass (g)
Cypriniformes	Catostomidae	*Catostomus commersoni*	White sucker	Omnivore	Cycloid	4	Minnesota	307–430	12–261
	Cyprinidae	*Pimphales notatus*	Bluntnose minnow	Omnivore	Cycloid	4	Minnesota, Michigan	51–58	0·23–0·38
		P. promelas	Fathead minnow	Omnivore	Cycloid	11	Iowa, Minnesota	35–58	0·05–0·4
		Rhinichthys cataractae	Longnose dace	Insectivore	Cycloid	4	Minnesota	62–100	0·5–3·2
		Notemigonus crysoleucas	Golden shiner	Omnivore	Cycloid	3	Iowa	107–123	2·5–4·9
Esociformes	Esocidae	*Esox lucius*	Pike	Piscivore	Cycloid	10	Minnesota	325–470	40·2–146
Gasterosteiformes	Gasterosteidae	*Cluaea inconstans*	Brook stickleback	Insectivore	No true scales	2	Iowa	39–49	0·13–0·23
Perciformes	Percidae	*Perca flavescens*	Yellow perch	Piscivore	Ctenoid	13	Iowa, Minnesota	33–235	0·05–36
		Stizostedion vitreum	Walleye	Piscivore	Ctenoid	13	Minnesota	174–477	11–284
	Centrarchidae	*Pomoxis nigromaculatus*	Black crappie	Piscivore	Ctenoid	3	Minnesota	148–272	9–86
		Lepomis macrochirus	Bluegill	Insectivore	Ctenoid	43	Minnesota, Michigan	36·5–171	0·16–22
		Lepomis gibbosus	Pumpkinseed	Insectivore	Ctenoid	10	Minnesota, Michigan	81–181	2·3–38
		Micropterus salmoides	Largemouth bass	Piscivore	Ctenoid	10	Minnesota	225–290	5·3–100
		M. dolomieu	Smallmouth bass	Piscivore	Ctenoid	3	Minnesota	186–321	20–157
		Ambloplites rupestris	Rock bass	Insectivore	Ctenoid	9	Minnesota	50–216	15–47

TABLE I. Continued

Order	Family	Genus and species	Common name	Feeding type	Scale type	Sample size	Sampling locations	Range L_T (mm)	Range Dry mass (g)
Salmoniformes	Salmonidae	*Oncorhynchus mykiss*	Rainbow trout	Planktivore	Cycloid	2	Minnesota	295–296	59–82
		Coregonus sp.	Whitefish	Planktivore	Cycloid	5	Minnesota	224–343	34–161
Siluriformes	Ictaluridae	*Ameiurus melas*	Black bullhead	Omnivore	Scaleless	7	Minnesota	158–340	8·7–157
		A. natalis	Yellow bullhead	Omnivore	Scaleless	3	Minnesota	147–353	5·5–128
		A. nebulosus	Brown bullhead	Omnivore	Scaleless	4	Minnesota	183–305	17–81

© 2007 The Authors

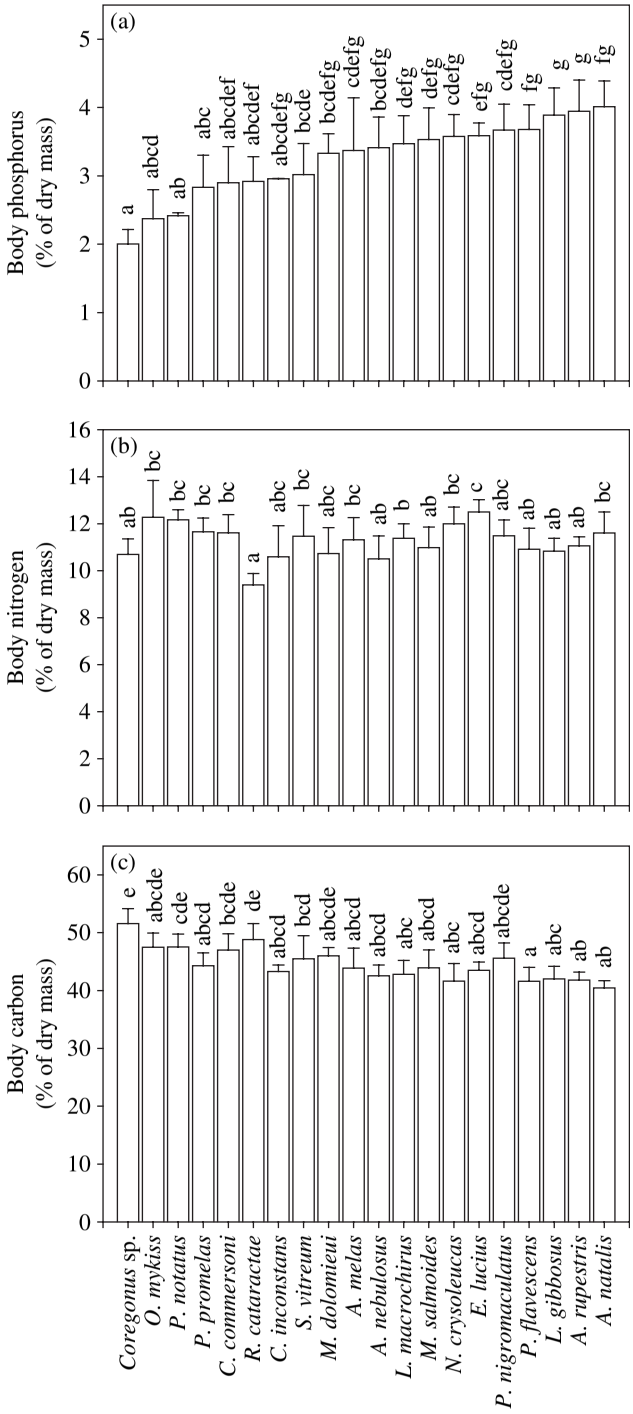

FIG. 1. Mean ± S.D. whole-body nutrient content of 20 fish species arranged from lowest to highest % P for (a) phosphorus, (b) nitrogen and (c) carbon (see Table I for sample sizes and names). Similar lowercase letters above bars indicate homogeneous groups as determined by Tukey's HSD test.

© 2007 The Authors

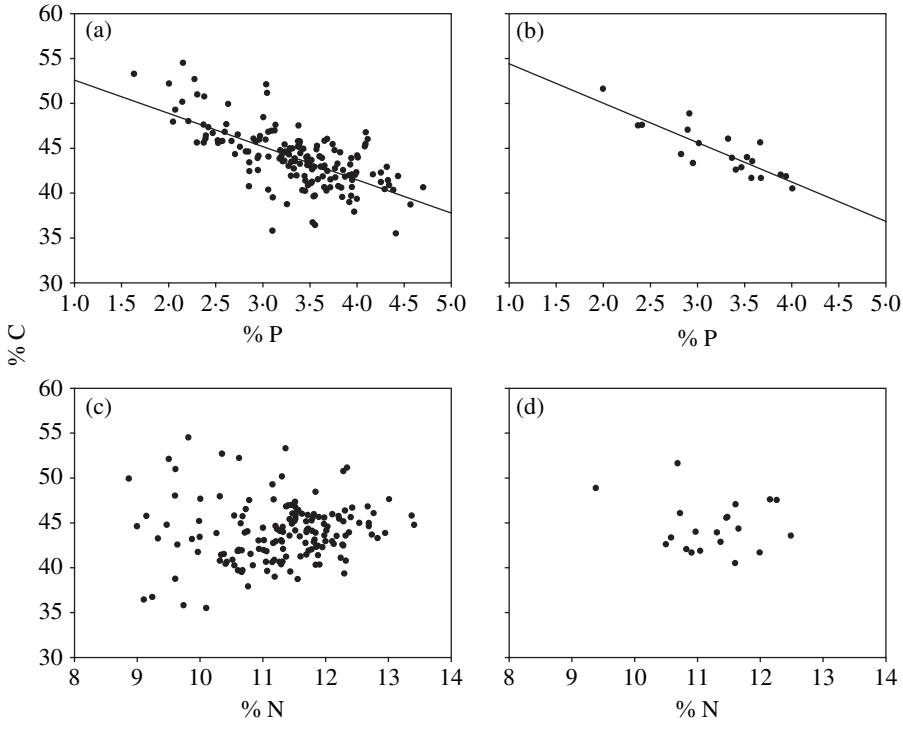

FIG. 2. Stoichiometric covariation in whole-body elemental content. (a), (b) Carbon and phosphorus and (c), (d) carbon and nitrogen for (a), (c) all individuals and (b), (d) species means. The curve in (a) was fitted by $y = 56{\cdot}3 + 3{\cdot}7x$ ($r^2 = 0{\cdot}44$, $P < 0{\cdot}001$) and in (b) by $y = 58{\cdot}8 - 4{\cdot}4x$ ($r^2 = 0{\cdot}73$, $P < 0{\cdot}001$). % C did not vary significantly with % N.

was unrelated to P and C content. These relationships were also not significant in phylogenetically corrected analyses (N *v.* P: $r = 0{\cdot}36$, $P > 0{\cdot}05$; N *v.* C: $r = 0{\cdot}425$, $P > 0{\cdot}05$). As shown below, Ca may account for some though not all of this remaining mass.

There was also considerable variation in the stoichiometric ratios C:P, N:P and C:N among fish species (Fig. 3). All three nutrient ratios showed significant variation across species (N:P $F_{19,137}$, $P < 0{\cdot}001$; C:P $F_{19,137}$, $P < 0{\cdot}001$; C:N $F_{19,137}$, $P < 0{\cdot}001$). Not unexpectedly, N:P was low in high-P fishes, ranging from *c.* 6 in centrarchids [*Pomoxis nigromaculatus* (Lesueur), *Lepomis gibbosus* (L.) and *Ambloplites rupestris* (Rafinesque)] to *c.* 12 in salmonids [*Coregonus clupeaformes* (Mitchill) and *O. mykiss*] [Fig. 3(b)]. With increasing P, C:P also decreased [Fig. 3(a)]. Again, species from the Salmonidae (*C. clupeaformes* and *O. mykiss*), Cyprinidae [*Pimephales notatus* (Rafinesque), *Pimephales promelas* Rafinesque and *Rhinichthys cataractae* (Valenciennes)] and Catostomidae [*Catostomus commersoni* (Lacepède)] families had high C:P while species from the Centrarchidae [*P. nigromaculatus*, *Perca flavescens* (Mitchill), *L. gibbosus* and *A. rupestris*], Esocidae (*Esox lucius* L.), and Ictaluridae [*Ameiurus nebulosus* (LeSuer) and *Ameiurus natalis* (LeSuer)] families had low C:P. There is less variation evident in C:N, although higher C:N appears associated with higher P. Tests for phylogenetic

© 2007 The Authors

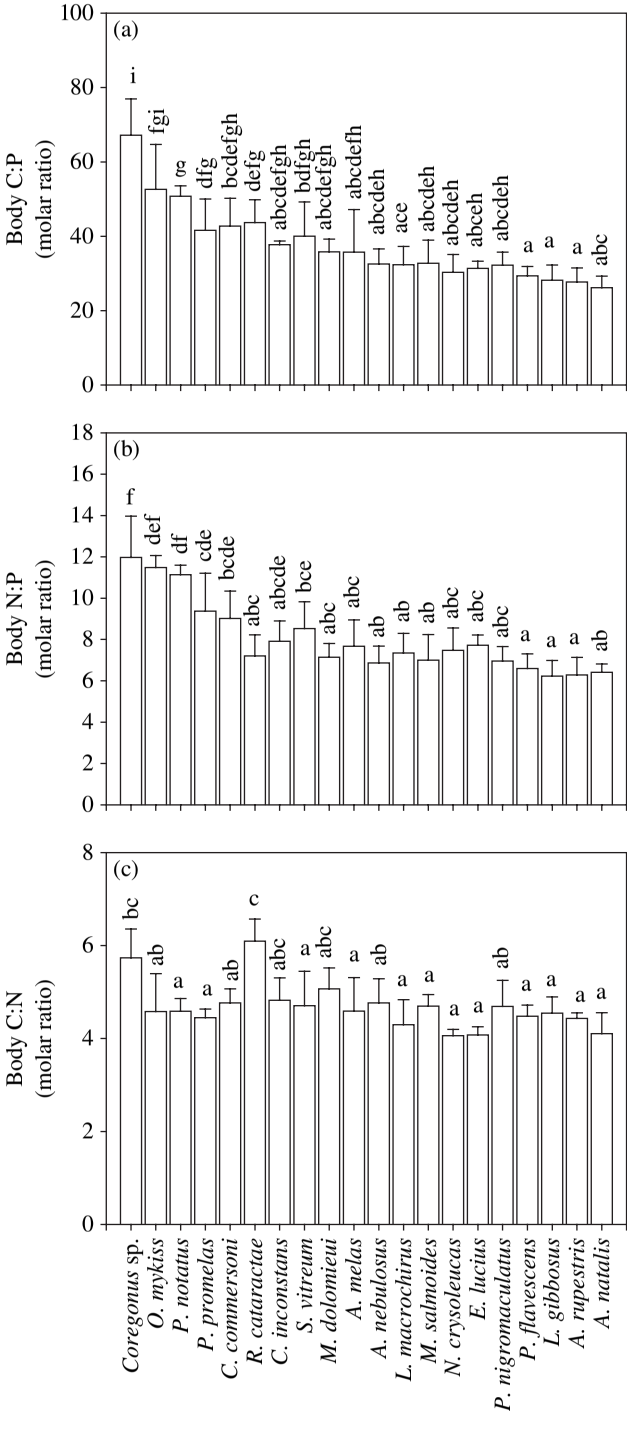

FIG. 3. Mean ± S.D. whole-body stoichiometric ratios (mol:mol) of 20 fish species arranged from lowest to highest % P for (a) C:P, (b) N:P and (c) C:N. Similar lowercase letters above bars indicate homogeneous groups as determined by Tukey's HSD test.

© 2007 The Authors

signals were significant for C:P ($K = 0{\cdot}677$, $P < 0{\cdot}01$) and N:P ($K = 0{\cdot}832$, $P < 0{\cdot}01$) but not for C:N ($K = 0{\cdot}314$, $P > 0{\cdot}05$).

STOICHIOMETRY AND BONY MATTER

Fish bone averaged 25·3% Ca and 11·8% P. The mean Ca:P in bone samples was 2·14 (by mass), which is nearly identical to the 2·15 ratio found by Russell *et al.* (1986) for pure hydroxyapatite salt, the major mineral found in bone, calcified cartilage, dentine and enamel. There was a strikingly high degree of correlation between these two elements in whole fishes, with the slope of 2·3 being very close to the Ca:P ratio measured in bone (Fig. 4). The correlation observed between Ca and P was the highest of any pair of any elements examined. The high correlation between Ca and P implies that most of the stoichiometric variation P content has a structural, mineralogical basis relating to varying degrees of boniness in skeleton and integument.

To evaluate further the role of structural P (both in bones and in scales), an estimate of the maximum contribution of bone to fish whole-body P was calculated by assuming all fish Ca was associated with bone. Though this assumption is known to be false, it is the best available way to estimate the structural P pool, and it is expected that any inaccuracy introduced because of non-skeletal Ca might be relatively constant across fish species. The fit was extrapolated to zero Ca content and the % P not associated with Ca (0·90%) was subtracted from the observed whole fish P contents. These maximal estimates ranged from slightly less than half to more than three-quarters of fish P associated with structural, bony matter (mean = 72·7%, range 46·3–81·3%).

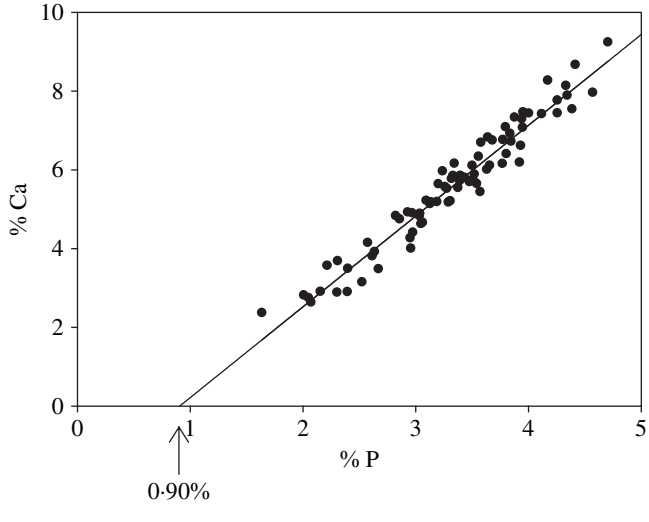

FIG. 4. Whole fish % Ca and % P in 18 species. The curve is extrapolated to zero Ca content to estimate the P not associated with Ca and was fitted by $y = 2{\cdot}31x - 2{\cdot}09$ ($r^2 = 0{\cdot}95$).

© 2007 The Authors

ALLOMETRY

Nine species with sample sizes greater than six were tested for allometric stoichiometric trends, and several significant stoichiometric allometries were found (Table II). Intraspecific relationships varied among species, with allometries for individual chemical parameters occasionally differing from positive to negative between species. A weak relationship between bluebill % P and L_T was factored into the measurement of habitat-specific stoichiometry (below).

Interspecific allometry (Fig. 5) suggested an interesting and unexpected pattern: individuals or species of intermediate size had highest P and were lowest in N:P. There were significant quadratic trends for P and N:P both for all individuals and for species means (Fig. 5). Neither fish P concentration ($n = 19$, $r^2 = 0{\cdot}0003$, $P > 0{\cdot}05$) nor the N:P ratio ($n = 19$, $r^2 = 0{\cdot}024$, $P > 0{\cdot}05$), however, were significantly related to L_T after controlling for phylogeny. Representatives of the salmonid and cyprinid lineages strongly influenced the allometric trends [Fig. 5(b), (d)] and statistical power to relate stoichiometry to fish size was consequently reduced in a phylogenetically informed analysis. Thus, statistical evidence for stoichiometric allometry is somewhat ambiguous and more of the phylogenetic tree would need to be sampled to obtain more definitive results.

HABITAT SPECIFICITY

Bluegill was the species sampled most intensively in the largest number of lakes. Individuals from 12 lakes were used to test for habitat-specific differences in body P content. Bluegill P content ranged *c.* 1·7% (from 2·71 to 4·42%; $F_{11,27}$, $P < 0{\cdot}001$) across lakes when L_T was not taken into account [Fig. 6(a)]. A weak positive allometry between P content and L_T was then factored out [% P $= 3{\cdot}04 + 0{\cdot}005\ L_T$, $r^2 = 0{\cdot}10$, $P = 0{\cdot}051$; Fig. 6(b)]. Differences among lakes were then reduced, with still about a 1% P range from the highest to the lowest populations. Other factors such as sex or season cannot be factored out in affecting stoichiometry, so this 1% P variation may represent a maximum estimate of the purely 'environmental' variation in this sample.

TABLE II. Significant allometric trends from the nine species of fishes tested (see Table I). Not all species had significant allometric trends

	Species	Slope	r^2	P
%C	Black bullhead	0·04 (0·012)	0·79	0·007
	Bluegill	−0·03 (0·014)	0·11	0·04
% N	Black bullhead	−0·012 (0·003)	0·80	0·006
	Bluegill	−0·012 (0·003)	0·25	0·001
% P	Black bullhead	−0·009 (0·003)	0·70	0·018
N:P	Black bullhead	0·014 (0·005)	0·60	0·042
	Bluegill	−0·016 (0·005)	0·18	0·007
C:P	Black bullhead	0·15 (0·05)	0·79	0·008
	Walleye	−0·063 (0·028)	0·32	0·043
C:N	Black bullhead	0·010 (0·001)	0·91	<0·001
	Yellow perch	0·002 (0·001)	0·43	0·015

© 2007 The Authors
Journal compilation © 2007 The Fisheries Society of the British Isles,

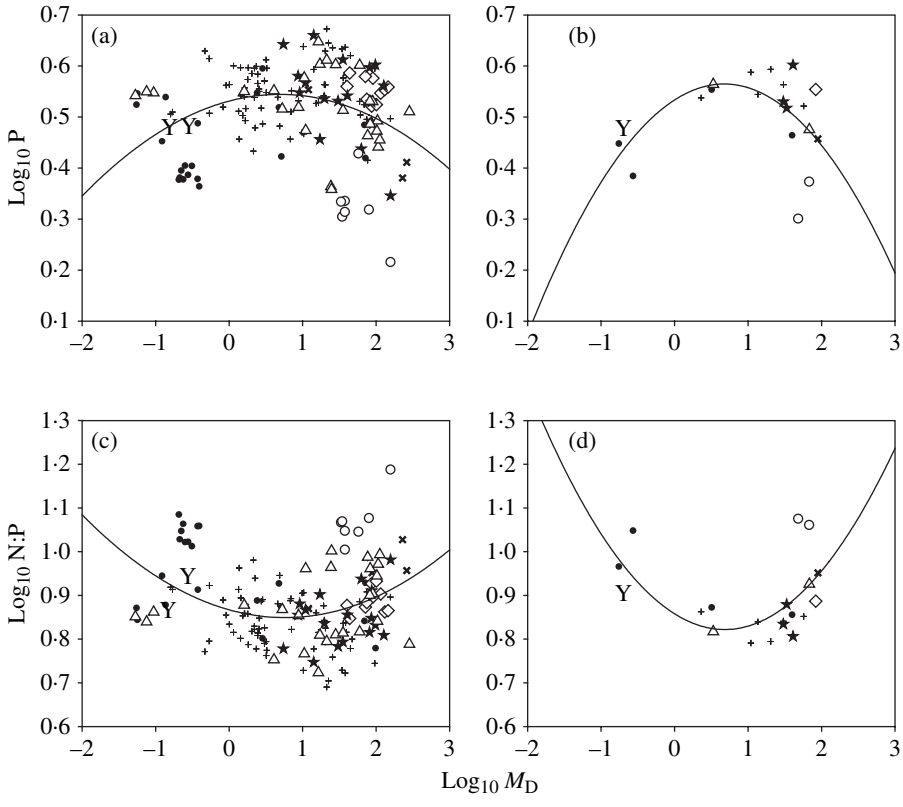

FIG. 5. The allometry of P and N:P stoichiometry in the families: Ictaluridae (★), Salmonidae (○), Centrarchidae (+), Cyprinidae (●), Esocidae (◇), Gasterosteidae (Y), Percidae (△) and Catostomidae (✖). (a), (b) P and dry mass (M_D) and (c), (d) N:P and M_D for (a), (c) individual specimens and (b), (d) species means. The curves were fitted: (a) $y = 0{\cdot}531 + 0{\cdot}0381x - 0{\cdot}0275x^2$ ($r^2 = 0{\cdot}09$, $P < 0{\cdot}001$), (b) $y = 0{\cdot}533 + 0{\cdot}0928x - 0{\cdot}0687x^2$ ($r^2 = 0{\cdot}31$, $P < 0{\cdot}05$), (c) $y = 0{\cdot}0867 - 0{\cdot}0470x + 0{\cdot}0309x^2$ ($r^2 = 0{\cdot}10$, $P < 0{\cdot}01$) and (d) $y = 0{\cdot}858 - 0{\cdot}106x + 0{\cdot}0773x^2$ ($r^2 = 0{\cdot}35$, $P < 0{\cdot}05$).

DISCUSSION

Fishes are amenable to stoichiometric analysis; evidence indicates that they show great interspecific and lesser intraspecific variation. Strict homeostasis (constancy of organism elemental content in the face of chemically varying food resources) is a simplifying assumption about a more complex reality, where nutrient content varies with many factors including the chemical content of food ingested. Theoretical studies in ecological stoichiometry often treat elemental content as a taxon-specific trait, and when this is an appropriate assumption, stoichiometric principles can be used to connect species traits to ecosystem processes (Sterner & Elser, 2002). Thus, the general partitioning of variance in element content within and across species is critical.

The way that variation in elemental stoichiometry relates to phylogeny is only now beginning to be revealed. The *K* statistic (Blomberg *et al.*, 2003) reflects the way that a trait maps onto a taxonomic tree. If phenotypic variation is distributed randomly across the tips of a phylogenetic tree, the *K* statistic will be low.

© 2007 The Authors

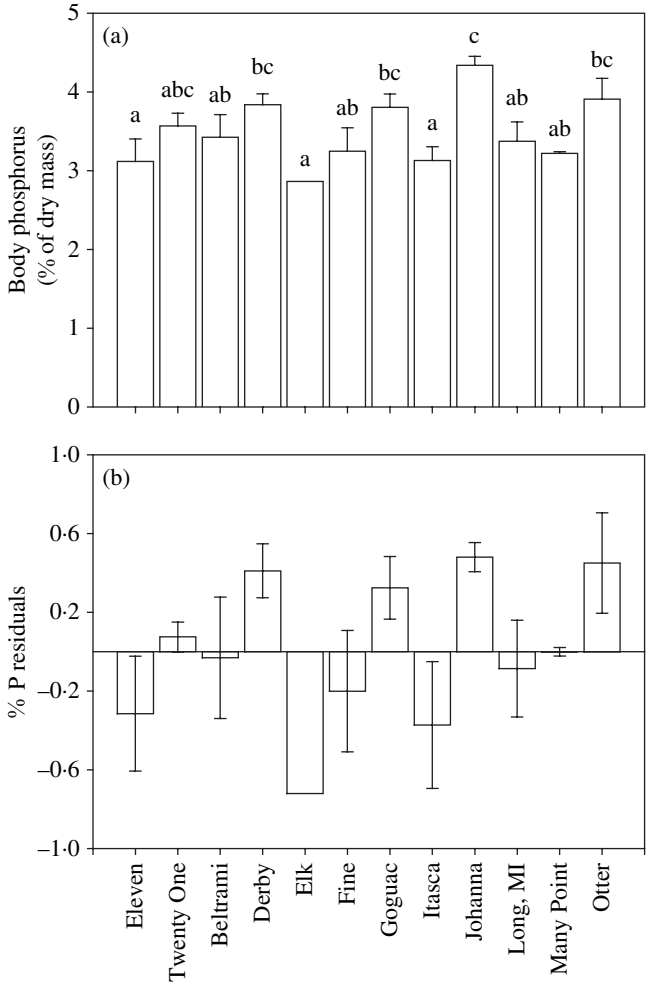

FIG. 6. Intraspecific P content as a function of habitat. (a) Bluegill mean ± S.D. % P for each of 12 lakes. Similar lowercase letters above bars indicate homogeneous groups as determined by Tukey's HSD test. (b) Residual variation ± S.D. after taking a weak P allometry into account.

Where variation is deeply rooted (ancient in origin), so that species within phylogenetic clades are very similar to each other, K will be high. K exhibits desirable statistical properties of good power and insensitivity to sample size (for sample sizes >20). Stoichiometric parameters involving fish P were found to have values of K in the range of 0·68–0·83; all these were statistically significant. Stoichiometric parameters involving only C or N were lower and were not statistically significant. Blomberg *et al.* (2003) calculated average K for studies of traits reflecting body size (0·83), morphology (0·71), life history (0·63), physiology (0·54) and behaviour (0·35). Fish P has similar taxonomic 'rootedness' as such morphological factors as body size and morphology. This correspondence could stem from the use of P in structural matter, which will tie it closely to body size and morphology.

© 2007 The Authors
Journal compilation © 2007 The Fisheries Society of the British Isles,

Bluegill P content, among 43 individuals within 12 lakes, was found to be significantly habitat-specific. Not quite one-half of this variation (1% out of 1·7%) was attributable to allometry. The remaining variation might relate to sex, season, genetically distinct populations or other life-history parameters. Some of it probably also relates to feeding history (*e.g.* fish condition) and would thus represent departure from strict stoichiometric homeostasis. Without experimental feeding trials, how much of this variation is purely environmental cannot be determined. Fish stoichiometry has a large phylogenetically based interspecific component, and a smaller intraspecific component related to life-history and environmental factors.

Low-P fish species (left half of Figs 1 and 2) tend to be soft-rayed fishes with cycloid scales belonging to the Cyprinidae and Salmonidae. These fishes are also generally more streamlined and are elongate in shape. High-P species (right half of Figs 1 and 2) tend to be spiny-rayed fishes with ctenoid scales belonging to the Centrarchidae and Percidae. The majority of these high-P fishes are laterally compressed. One exception is moderate- to high-P Ictaluridae, which are not laterally compressed and also have no scales, but have heavily ossified craniums. Moderate to low-P Salmonidae have incompletely ossified craniums, much more cartilage and less bone than many other fishes. The degree of ossification of the internal skeleton and the outer integument thus both contribute to whole fish stoichiometric patterns.

Differences in P content may also be attributed to differences in feeding guild. Four feeding guilds, planktivores, omnivores, piscivores and insectivores, were assigned to the 20 species of fishes based on the dominant food type consumed by adults (Becker, 1983). Phosphorus content was found to be significantly different among the guilds using a straightforward one-way ANOVA (Fig. 7). A phylogenetically corrected analysis, however, casts doubt on the interpretation of these differences. After running a Monte-Carlo simulation of character evolution along the specific phylogeny, then running separate ANOVAs on results from each run of the simulation to find the critical F-value, the results were no longer significant. Feeding guild is strongly related to phylogeny, so the non-independence of the observations of individual species plays a role in the analysis. It is therefore difficult to confidently conclude that feeding guild affects species stoichiometry, but the results are suggestive.

The results of this study are consistent with previous studies, discussed earlier, showing taxon-specific allometric patterns in fish element stoichiometry. For example, N:P increased with size in black bullheads *Ameiurus melas* (Rafinesque), indicating that the N:P of new tissue added during growth is greater than the N:P of whole small black bullheads. The opposite allometric trend, however, was seen in bluegill: the N:P of new tissue was lower than the N:P of whole small bluegill. Allometric patterns involving C were also different in black bullhead and bluegill. High C:N may be caused by high lipids or high carbohydrate content (Gnaiger & Bitterlich, 1984). Black bullheads had increasing C with L_T, indicating they probably became more lipid rich with length while bluegill probably became more lipid poor with length.

A single allometry for N:P also was not found across species. Across species, the highest P content and lowest N:P ratio were observed in fishes of intermediate size [the shift in allometric trends occurred at *c.* 10 g mass (Fig. 5)].

© 2007 The Authors

Perhaps the aforementioned differences within black bullheads and bluegill relate to these global trends because bluegill are generally in the lower range of the body sizes where N:P decreases with size but black bullheads are generally in the higher range where N:P increases with size. This quadratic curve observed over four orders of magnitude of fish body mass is opposite in direction to the broad-scale relationship spanning unicells to whales hypothesized by Elser *et al.* (1996). A phylogenetically informed analysis, however, complicates the interpretation of these interspecific patterns, similar to what was found for feeding guild. When accounting for the phylogeny, interspecific allometric trends no longer were statistically significant. Therefore, even though fish P content and N:P ratio were significantly related to body mass, the possibility cannot be ruled out that these patterns are driven by other factors held in common among taxa because of a shared evolutionary history.

Again, because fish mass is deeply rooted in the phylogeny, there is a non-independence of the observations of individual species, and evolutionary selective forces on body mass and nutrient stoichiometry cannot be confidently connected with this sample. Phosphorus concentration appears to be a fundamental characteristic of species as evidenced by the 'tight' correlation of body P with phylogeny, and could be a leading factor in the diversification of fishes. The strong phylogenetic signal for P suggests phylogenetically informed analyses are essential for evaluating hypotheses concerning the evolution of stoichiometry in fishes. Specific causes of differing P concentrations cannot be inferred based on patterns seen with size or feeding type because related species may have similar traits. More data would be needed to conduct phylogenetic analyses on traits that are closely related to phylogeny. As a predictive tool,

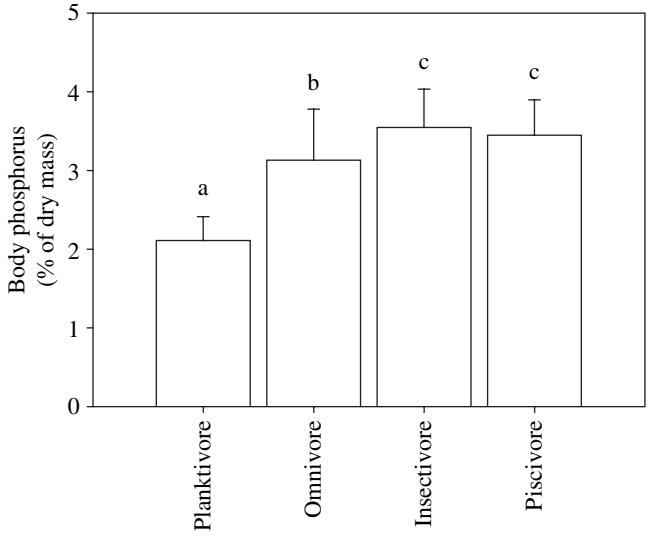

FIG. 7. Phosphorus stoichiometry as a function of feeding type (values are means ± S.D.). Similar lowercase letters above bars indicate homogeneous groups as determined by Tukey's HSD test on species means uncorrected for phylogeny.

© 2007 The Authors

however, the traditional (non-phylogenetic) analyses indicate that fishes of intermediate size, or piscivorous or insectivorous fishes, are generally P-rich.

Ecological stoichiometry is concerned with linkages among elements. Where elements are tightly linked, biogeochemical cycling of one is closely coupled to the other. The close correspondence observed in fish Ca and P (Fig. 4), besides strongly implicating skeletal (structural) factors in accounting for the considerable variation in fish P content, also indicates that Ca and P cycling through fishes in aquatic ecosystems are tightly linked. Linkages such as these might be exploited to simplify the otherwise highly multi-dimensional biogeochemical system of many elements distributed among many species.

The present study thus contributes to a deeper understanding of the evolutionary basis for stoichiometric patterns in fishes. It also helps to resolve macro-ecological patterns in nutrients within aquatic ecosystems.

The authors would like to thank T. Garland for helping with the PHYSIG programme and J. Elser and B. Harvey for helpful comments and suggestions with this paper. The study was funded by NSF grant DEB-9815597 and DEB-9977047.

References

Arendt, J., Wilson, D. S. & Stark, E. (2001). Scale strength as a cost of rapid growth in sunfish. *Oikos* **93,** 95–100.

Attayde, J. L. & Hansson, L. A. (2000). Fish-mediated nutrient recycling and the trophic cascade in lakes. *Canadian Journal of Fisheries and Aquatic Sciences* **58,** 1924–1931.

Becker, G. C. (1983). *Fishes of Wisconsin*. Madison, WI: University of Wisconsin Press.

Blomberg, S. P., Garland, T. Jr & Ives, A. R. (2003). Testing for phylogenetic signal in comparative data: behavioral trains are more labile. *Evolution* **57,** 717–745.

Brabrand, A., Faafeng, B. & Nilssen, J. P. (1990). Relative importance of phosphorus supply to phytoplankton production: fish versus external loading. *Canadian Journal of Fisheries and Aquatic Sciences* **47,** 364–372.

Cassadevall, M., Casinos, A., Viladiu, C. & Ontañon, M. (1990). Scaling of skeletal mass and mineral content in teleosts. *Zoologischer Anzeiger* **225,** 144–150.

Cavender, T. M. & Coburn, M. M. (1991). Phylogenetic relationships of North American cyprinidae. In *Systematics, Historical Ecology, and North American Freshwater Fishes* (Mayden, R. L., ed.), pp 293–327. Stanford, CA: Stanford University Press.

Coburn, M. M. & Cavender, T. M. (1991). Interrelationships of North American cyprinid fishes. In *Systematics, Historical Ecology, and North American Freshwater Fishes* (Mayden, R. L., ed.), pp. 328–373. Stanford, CA: Stanford University Press.

Davis, J. A. & Boyd, C. E. (1978). Concentrations of selected elements and ash in bluegill (*Lepomis macrochirus*) and certain other freshwater fish. *Transactions of the American Fisheries Society* **107,** 862–867.

Elser, J. J., Dobberfuhl, D., MacKay, N. A. & Schampel, J. H. (1996). Organism size, life history, and N:P stoichiometry: towards a unified view of cellular and ecosystem processes. *BioScience* **46,** 674–684.

Elser, J. J., Acharya, K., Kyle, M., Cotner, J., Makino, W., Markow, T., Watts, T., Hobbie, S., Fagan, W., Schade, J., Hood, J. & Sterner, R. W. (2003). Growth rate – stoichiometry couplings in diverse biota. *Ecology Letters* **6,** 936–943.

Fagan, W. F., Siemann, E., Mitter, C., Denno, R. F., Huberty, A. F., Woods, H. A. & Elser, J. J. (2002). Nitrogen in insects: implications for trophic complexity and species diversification. *The American Naturalist* **160,** 784–802.

Felsenstein, J. (1985). Phylogenies and the comparative method. *The American Naturalist* **125,** 1–15.

Fink, S. V. & Fink, W. L. (1981). Interrelationships of the ostariophysan fishes. *Zoological Journal of the Linnean Society* **72,** 297–353.

© 2007 The Authors

Garland, T. Jr, Harvey, P. H. & Ives, A. R. (1992). Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Systematic Botany* **41,** 18–32.
Gillooly, J. F., Allen, A. P., Brown, J. H., Elser, J. J., Martinez del Rio, C., Savage, V. M., West, G. B., Woodruff, W. H. & Woods, H. A. (2005). The metabolic basis of whole-organism RNA and phosphorus content. *Proceedings of the National Academy of Sciences* **102,** 11923–11927.
Gnaiger, E. & Bitterlich, G. (1984). Proximate biochemical composition and caloric content calculated from elemental CHN analysis: a stoichiometric concept. *Oecologia* **62,** 289–298.
Goodyear, C. P. & Boyd, C. E. (1972). Elemental composition of largemouth bass (*Micropterus salmoides*). *Transactions of the American Fisheries Society* **101,** 545–547.
Hecky, R. E. & Hesslein, R. H. (1995). Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. *Journal of the North American Benthological Society* **14,** 631–653.
Hendrixson, H. A. (2002). Stoichiometry of freshwater fish in relation to allometry and phylogeny, and the role of skeleton in fish stoichiometry. MS Thesis, University of Minnesota, St Paul, MN.
Hjerne, O. & Hansson, S. (2002). The role of fish and fisheries in Baltic Sea nutrient dynamics. *Limnology and Oceanography* **47,** 1023–1032.
Hood, J. M., Vanni, M. J. & Flecker, A. S. (2005). Nutrient recycling by two phosphorus-rich grazing catfish: the potential for phosphorus-limitation of fish growth. *Oecologia* **146,** 247–257.
Johnson, G. D. & Patterson, C. (1996). Relationships of lower euteleostean fishes. In *Interrelationships of Fishes* (Stiassny, M. L. J., Parenti, L. R. & Johnson, G. D., eds), pp. 251–332. San Diego, CA: Academic Press.
Kay, A. D., Ashton, I. W., Gorokhova, E., Kerkhoff, A. J., Liess, A. & Litchman, E. (2005). Toward a stoichiometric framework for evolutionary biology. *Oikos* **109,** 6–17.
Kitchell, J. F., Koonce, J. F. & Tennis, P. S. (1975). Phosphorus flux through fishes. *Internationale Vereinigung für theoretische und angewandte Limnologie Verhandlungen* **19,** 2478–2484.
Kraft, C. E. (1992). Estimates of phosphorus and nitrogen cycling by fish using a bioenergetics approach. *Canadian Journal of Fisheries and Aquatic Sciences* **49,** 2596–2604.
Lauder, G. V. & Liem, K. F. (1983). The evolution and interrelationships of the actinopterygian fishes. *Bulletin of the Museum of Comparative Zoology* **150,** 95–197.
Lundberg, J. G. (1970). The evolutionary history of North American catfishes, family Ictaluridae. PhD Thesis, University of Michigan.
Moyle, P. B. & Cech, J. J. (2004). *Fishes. An Introduction to Ichthyology*. Upper Saddle River, NJ: Prentice Hall.
Nakashima, B. S. & Leggett, W. C. (1980). The role of fishes in the regulation of phosphorus availability in lakes. *Canadian Journal of Fisheries and Aquatic Sciences* **37,** 1540–1549.
Parmenter, R. R. & Lamarra, V. A. (1991). Nutrient cycling in a freshwater marsh: the decomposition of fish and waterfowl carrion. *Limnology and Oceanography* **36,** 976–987.
Penczak, T. (1985). Phosphorus, nitrogen, and carbon cycling by fish populations in two small lowland rivers in Poland. *Hydrobiologia* **120,** 159–165.
Peters, R. H. (1983). *The Ecological Implications of Body Size*. Cambridge: Cambridge University Press.
Prange, H. D., Anderson, J. F. & Rahn, H. (1979). Scaling of skeletal mass to body mass in birds and mammals. *The American Naturalist* **113,** 103–122.
Russell, R. G. G., Caswell, A. M., Hearn, P. R. & Sharrard, R. M. (1986). Calcium in mineralized tissues and pathological calcification. *British Medical Bulletin* **42,** 435–446.

© 2007 The Authors
Journal compilation © 2007 The Fisheries Society of the British Isles,

Schaus, M. H., Vanni, M. J. & Stein, R. A. (1997). Nitrogen and phosphorus excretion by detritivorous gizzard shad in a reservoir ecosystem. *Limnology and Oceanography* **42,** 1386–1397.
Schindler, D. E. & Eby, L. A. (1997). Stoichiometry of fishes and their prey: implications for nutrient recycling. *Ecology* **78,** 1816–1831.
Siebert, D. J. (1987). Interrelationships among families of the order Cypriniformes (Teleostei). PhD Thesis, City University of New York.
Sterner, R. W. (1990). The ratio of nitrogen to phosphorus resupplied by herbivores: zooplankton and the algal competitive arena. *The American Naturalist* **136,** 209–229.
Sterner, R. W. & Elser, J. J. (2002). *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*. Princeton, NJ: Princeton University Press.
Sterner, R. W. & George, N. B. (2000). Carbon, nitrogen and phosphorus stoichiometry of cyprinid fishes. *Ecology* **81,** 127–140.
Tang, H. J. & Xie, P. (2000). Budgets and dynamics of nitrogen and phosphorus in a shallow, hypereutrophic lake in China. *Journal of Freshwater Ecology* **15,** 505–514.
Tanner, D. K., Brazner, J. C. & Brady, V. J. (2000). Factors influencing carbon, nitrogen, and phosphorus content of fish from a Lake Superior coastal wetland. *Canadian Journal of Fisheries and Aquatic Sciences* **57,** 1243–1251.
Tatrai, I. (1987). Role of fish and benthos in the nitrogen budget of Lake Balaton, Hungary. *Archiv für Hydrobiologie* **110,** 291–302.
Threlkeld, S. T. (1988). Planktivory and planktivore biomass effects on zooplankton, phytoplankton, and the trophic cascade. *Limnology and Oceanography* **33,** 1362–1375.
Vanni, M. J. & Findlay, D. L. (1990). Trophic cascades and phytoplankton community structure. *Ecology* **71,** 921–937.
Vanni, M. J., Layne, C. D. & Arnott, S. E. (1997). ''Top-down'' trophic interactions in lakes: effects of fish on nutrient dynamics. *Ecology* **78,** 1–20.
Vanni, M. J., Flecker, A. S., Hood, J. M. & Headworth, J. L. (2002). Stoichiometry of nutrient recycling by vertebrates in a tropical stream: linking species identity and ecosystem processes. *Ecology Letters* **5,** 285–293.
Williams, R. J. P. & Fraústo da Silva, J. J. R. (1996). *The Natural Selection of the Chemical Elements: The Environment and Life's Chemistry*. Oxford: Clarendon Press.
Woods, H. A., Fagan, W., Elser, J. J. & Harrison, J. F. (2004). Allometric and phylogenetic variation in insect phosphorus content. *Functional Ecology* **18,** 103–109.

© 2007 The Authors
Journal compilation © 2007 The Fisheries Society of the British Isles,