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# Intra- and interspecific differences in nutrient recycling by European freshwater fish

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## SUMMARY

1. We measured N and P excretion rates of 470 individuals belonging to 18 freshwater fish species widespread in Western Europe. We assessed the effect of body mass on excretion rates at both the intra- and interspecific levels.
2. The high variability in *per capita* N and P excretion rates was mainly determined by differences in body mass. The scaling coefficients of allometric relationships for both N and P excretion rates were significantly lower than 1 (mean ± SE, 0.95 ± 0.04 and 0.81 ± 0.05, respectively).
3. The slope of the allometric relationship between fish mass and nutrient excretion rate was significantly different among species. We did not detect any influence of phylogenetic conservatism on fish mass and on excretion rates. Further investigations are needed to understand the biological determinants of these differences.
4. This high intra- and interspecific variability in *per capita* excretion rates, coupled with differences in fish body mass, produce marked differences in biomass-standardised excretion rates. These results thus indicate the necessity for further experimental and *in situ* investigations on the consequences of nutrient recycling by fish in freshwater ecosystems.

*Keywords*: excretion, nitrogen, phosphorus, phylogenetic conservatism, stoichiometry

## Introduction

One of the main issues in ecology is to understand better how biodiversity determines ecosystem processes, which ultimately provide ecosystem services to human populations. This task is of particular importance in a global change context (Diaz *et al*., 2006). For instance, freshwater ecosystems provide protein to human populations through fishing and aquaculture but also contribute significantly to the regulation of nutrient cycles (Costanza *et al*., 1997). Aimed at a better assessment of how biodiversity affects ecosystem functioning, a functional view of biological communities has emerged during the last decade (McGill *et al*., 2006; Violle *et al*., 2007). This methodological framework focusses on the biological traits of species rather than on their taxonomic identity to assess how species respond to environmental constraints (natural or anthropogenic) and how, in turn, they can affect their environment.

In freshwater ecosystems, fish often account for the major part of the animal biomass and thus play a key role in ecosystem processes (Holmlund & Hammer, 1999). Studies on the role of freshwater fish in nutrient cycles are often restricted to the top-down control they play in the food web (Kitchell *et al*., 1979; Schindler *et al*., 1993, 1997). For instance, zooplanktivorous fish can reduce the abundance of grazing zooplankton, which can subsequently lead to an increased biomass of phytoplankton and modified nutrient dynamics (Vanni, Layne & Arnott, 1997). However, in addition to this indirect impact on primary productivity, fish also have a direct influence on primary producers through nutrient recycling (Vanni, 2002; Schmitz, Hawlena & Trussell, 2010). Indeed, fish metabolism produces waste, particularly ammonia and phosphate, which are mainly excreted by the kidney and the gills (Wright, 1995). The nitrogen (N) and phosphorus (P) initially trapped in the organic matter of living or dead

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organisms are thus released as ions directly available for primary producers (Vanni, 2002). Recycling of N and P could have important functional implications, since these nutrients often limit primary production in freshwater ecosystems (Elser *et al*., 2007). The role of fish in nutrient dynamics has long been considered negligible compared to microbial processes, although more recently many studies have shown that nutrient excretion by fish can contribute significantly to nutrient recycling (Tarvainen, Sarvala & Helminen, 2002; Vanni *et al*., 2006; McIntyre *et al*., 2008; Sereda *et al*., 2008b; Layman *et al*., 2011; Small *et al*., 2011) and can even create biogeochemical hotspots in low nutrient systems, that is, places where nutrient release by fish exceeds uptake by other organisms (McIntyre *et al*., 2008).

Previous studies have demonstrated the existence of strong differences in nutrient excretion by fish, in terms of both rates (molar amount excreted per fish per unit of time) and stoichiometry (N:P ratio) (Vanni, 2002; Vanni *et al*., 2002; Torres & Vanni, 2007; Sereda & Hudson, 2011; Small *et al*., 2011). The strong variability in *per capita* excretion rate is mainly because of the differences in body mass, with a global allometric relationship having a scaling coefficient lower than one (Vanni *et al*., 2002; Hall *et al*., 2007; McIntyre *et al*., 2008; Sereda, Hudson & McLoughlin, 2008a; Small *et al*., 2011). Nevertheless, beyond this general pattern, several studies have found interspecific differences in the effect of mass on excretion rates, that is, the parameters of the allometric relationship between mass and excretion rates differ between species (Hall *et al*., 2007; Torres & Vanni, 2007; McIntyre *et al*., 2008; Small *et al*., 2011). These differences in nutrient excretion rate have been related to differences in the ratio of nutrient concentrations in the diet and in the body (Vanni *et al*., 2002; Pilati & Vanni, 2007; Sereda *et al*., 2008a; Small *et al*., 2011). However, species that are phylogenetically close may tend to have more similar biological characteristics (e.g. size, diet, morphology, physiology) and thus similar nutrient excretion rates (Hendrixson, Sterner & Kay, 2007). Therefore, testing the influence of phylogeny on nutrient excretion rates remains a challenging issue (Hall *et al*., 2007; McIntyre & Flecker, 2010).

Almost all the assessments of both intra- and interspecific differences in nutrient recycling by fish have been carried out in North and South America (e.g. Vanni *et al*., 2002; Torres & Vanni, 2007; Verant *et al*., 2007; McIntyre *et al*., 2008; Sereda *et al*., 2008b; Small *et al*., 2011; but see Andre, Hecky & Duthie, 2003 and McIntyre *et al*., 2007 for studies on fish from the African Great lakes). Further investigations on phylogenetically different fish faunas are thus needed to determine whether the patterns of nutrient excretion rates are consistent across regions. In this study, we assessed nutrient excretion rates for 18 of the most common freshwater fish species in Western Europe. We then analysed the effect of mass on nutrient excretion at the intra- and interspecific level. We also tested whether interspecific differences in nutrient excretion rate are influenced by phylogenetic conservatism.

## Methods

### *Fish sampling*

We targeted 18 of the most common freshwater fish species in Western Europe (Table 1; Supporting Information Table S1). Fish sampling was conducted in the Garonne river basin (South Western France) in summer 2010 (between 22 June and 30 July). Sampling was conducted in four sites characterised by distinct habitat types and hence different species assemblages. All the individuals of each species came from the same site. In the Garbet River (Long 01°22′W; Lat 42°46′N, altitude 1100 m a.s.l.), a Pyrenean mountain tributary of the Garonne, we sampled typical upstream coldwater fish (water temperature, T = 10 °C during the sampling): the bullhead *Cottus gobio* and the brown trout *Salmo trutta*. The other sites were located at lower altitudes (ranging between 80 and 180 m a.s.l.) and hence water temperature during sampling was higher (T = 18–22 °C). In the Touch River (Long 01°13′W; Lat 43°29′N), a small lowland tributary of the Garonne, we sampled all the species typical of downstream habitats including both riffles and pools, namely the bleak *Alburnus alburnus*, the stone loach *Barbatula barbatula*, the barbel *Barbus barbus*, the gudgeon *Gobio gobio*, the common dace *Leuciscus leuciscus*, the toxostome *Parachondrostoma toxostoma*, the Eurasian minnow *Phoxinus phoxinus* and the chub *Squalius cephalus*. In the downstream part of the Tarn River (Long 01°19′W; Lat 44°01′N), one of the main tributaries of the Garonne, we targeted species from large lowland rivers, the European eel *Anguilla anguilla*, the white bream *Blicca bjoerkna* and the bitterling *Rhodeus amarus*. Finally, in three artificial lakes (gravel pits) around Toulouse city (Bidot lake, Long 01°17′W-Lat 43°31′N; Four de Louge lake, Long 01°18′W-Lat 43°26′N; Lamartine lake, Long 01°20′W-Lat 43°30′N), we sampled five species usually found in standing waters: the European perch *Perca fluviatilis*, the roach *Rutilus rutilus*, the rudd *Scardinius erythrophtalmus*, the black bullhead *Ameiurus melas* and the pumpkinseed *Lepomis gibbosus*. The two latter are non-native species introduced from North America, but frequently established in

**Table 1.** Effect of mass on nutrient excretion rate at the intraspecific level

| Species | | *n* | Mass (g) | SMA model $NH_4^+$ *c.* mass | | | SMA model $SRP^-$ *c.* mass | | | SMA model N:P *c.* mass | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Intercept | Slope | $R^2$ | Intercept | Slope | $R^2$ | Intercept | Slope | $R^2$ |
| *Alburnus alburnus* | Linnaeus 1758 | 21 | 12.0 ± 1.5 (3.2; 27.3) | 0.40 (0.20; 0.60) | 0.98 (0.81; 1.19) | **0.837** | −0.75 (−1.05; −0.45) | 0.94 (0.70; 1.27) | **0.602** | 0.70 (0.48; 0.93) | 0.48 (0.31; 0.74) | 0.118 |
| *Ameiurus melas* | Rafinesque 1820 | 25 | 49.4 ± 8.1 (9.1; 192.3) | 0.08 (−0.41; 0.57) | 1.011 (0.75; 1.36) | **0.516** | −1.10 (−1.57; −0.62) | 1.05 (0.79; 1.39) | **0.571** | 2.33 (1.79; 2.88) | −0.78 (−1.18; −0.51) | 0.008 |
| *Anguilla anguilla* | Linnaeus 1758 | 17 | 62.1 ± 19.2 (14.6; 332.0) | −0.02 (−0.32; 0.27) | 0.84 (0.68; 1.04) | **0.848** | −2.25 (−3.01; −1.49) | **1.54** (1.15; 2.08) | **0.703** | 2.57 (1.88; 3.25) | **−0.91** (−1.42; −0.59) | **0.321** |
| *Barbatula barbatula* | Linnaeus 1758 | 33 | 3.0 ± 0.2 (1.1; 5.8) | 0.38 (0.32; 0.45) | 0.91 (0.79; 1.06) | **0.836** | −0.73 (−0.82; −0.64) | 1.01 (0.84; 1.22) | **0.739** | 1.33 (1.22; 1.44) | −0.60 (−0.86; −0.42) | 0.003 |
| *Barbus barbus* | Linnaeus 1758 | 39 | 79.4 ± 14.4 (2.0; 331.7) | −0.70 (−0.95; −0.46) | **1.31** (1.17; 1.47) | **0.880** | −0.90 (−1.07; −0.72) | **0.72** (0.62; 0.84) | **0.801** | −0.05 (−0.31; 0.22) | **0.76** (0.62; 0.94) | **0.596** |
| *Blicca bjoerkna* | Linnaeus 1758 | 8 | 42.5 ± 24.9 (3.3; 174) | 0.48 (0.37; 0.59) | **0.85** (0.76; 0.94) | **0.989** | −0.69 (−1.03; −0.35) | 0.72 (0.50; 1.04) | **0.859** | 1.01 (0.74; 1.28) | 0.28 (0.14; 0.56) | 0.410 |
| *Cottus gobio* | Linnaeus 1758 | 18 | 7.3 ± 0.7 (3.6; 13.3) | 0.15 (−0.05; 0.34) | 0.82 (0.62; 1.08) | **0.716** | 1.16 (0.34; 1.97) | −1.83 (−3.01; −1.11) | 0.040 | −0.45 (−1.20; 0.31) | **1.97** (1.28; 3.05) | **0.287** |
| *Gobio gobio* | Linnaeus 1758 | 17 | 8.6 ± 1.3 (2.2; 20.5) | −0.63 (−0.78; −0.48) | 1.13 (0.97; 1.31) | **0.925** | −1.60 (−1.98; −1.23) | **1.69** (1.32; 2.16) | **0.798** | 1.23 (0.85; 1.61) | **−0.87** (−1.38; −0.55) | **0.237** |
| *Lepomis gibbosus* | Linnaeus 1758 | 18 | 13.2 ± 0.9 (6.8; 19.3) | 0.62 (0.27; 0.96) | 0.62 (0.38; 1.00) | 0.106 | −3.35 (−4.93; −1.76) | 2.89 (1.80; 4.64) | 0.136 | 4.62 (3.01; 6.24) | −2.87 (−4.67; −1.77) | 0.090 |
| *Leuciscus leuciscus* | Linnaeus 1758 | 5 | 194.3 ± 24.7 (126.4; 262.7) | −0.26 (−4.20; 3.69) | 1.08 (0.31; 3.77) | 0.242 | −4.31 (−8.49; −0.13) | 2.03 (0.90; 4.57) | 0.757 | 5.68 (1.02; 10.35) | −1.66 (−4.69; −0.59) | 0.550 |
| *Parachondrostoma toxostoma* | Vallot 1837 | 6 | 89.4 ± 16.8 (24.5; 152.3) | −0.11 (−0.72; 0.51) | 1.06 (0.78; 1.43) | **0.952** | −0.52 (−1.50; 0.46) | 0.61 (0.29; 1.31) | 0.639 | −0.17 (−1.54; 1.21) | 0.75 (0.32; 1.76) | 0.523 |
| *Perca fluviatilis* | Linnaeus 1758 | 15 | 74.7 ± 29.7 (13.9; 420.5) | 0.42 (0.22; 0.62) | **0.85** (0.73; 0.98) | **0.941** | −0.72 (−1.14; −0.31) | **0.61** (0.40; 0.91) | **0.517** | 0.65 (0.26; 1.04) | **0.55** (0.36; 0.83) | **0.492** |
| *Phoxinus phoxinus* | Linnaeus 1758 | 32 | 1.8 ± 0.1 (0.9; 4.0) | 0.36 (0.21; 0.52) | **1.94** (1.47; 2.56) | **0.436** | −0.68 (−0.82; −0.54) | **1.84** (1.41; 2.41) | **0.476** | 0.86 (0.77; 0.96) | 0.83 (0.58; 1.19) | 0.000 |
| *Rhodeus amarus* | Bloch 1782 | 21 | 2.7 ± 0.2 (1.3; 4.3) | 0.45 (0.35; 0.56) | 0.94 (0.73; 1.21) | **0.708** | −0.52 (−0.64; −0.40) | 0.90 (0.66; 1.22) | **0.563** | 0.73 (0.60; 0.86) | 0.64 (0.41; 1.01) | 0.035 |
| *Rutilus rutilus* | Linnaeus 1758 | 93 | 38.4 ± 2.7 (7.2; 176.7) | 0.59 (0.50; 0.68) | **0.71** (0.66; 0.78) | **0.844** | −0.76 (−0.97; −0.54) | 0.91 (0.78; 1.07) | **0.449** | 0.00 (−0.22; 0.22) | 0.69 (0.56; 0.85) | 0.004 |
| *Salmo trutta* | Linnaeus 1758 | 42 | 53.9 ± 9.3 (5.1; 241.9) | 0.57 (0.45; 0.69) | **0.75** (0.68; 0.83) | **0.901** | −1.18 (−1.33; −1.03) | 1.02 (0.93; 1.12) | **0.913** | 1.97 (1.81; 2.14) | **−0.42** (−0.53; −0.33) | **0.397** |
| *Scardinius erythrophtalmus* | Linnaeus 1758 | 21 | 39.2 ± 5.5 (13.2; 86.9) | 0.90 (0.72; 1.08) | **0.46** (0.36; 0.59) | **0.726** | −0.37 (−0.83; 0.10) | 0.63 (0.39; 0.99) | 0.001 | −0.22 (−0.75; 0.32) | 0.81 (0.54; 1.23) | **0.202** |
| *Squalius cephalus* | Linnaeus 1758 | 39 | 139.7 ± 33.8 (1.8; 1092) | −0.58 (−0.91; −0.24) | 1.21 (1.04; 1.40) | **0.795** | −1.51 (−1.87; −1.15) | 0.94 (0.76; 1.16) | **0.606** | −0.07 (−0.57; 0.43) | 0.86 (0.64; 1.16) | **0.160** |

Number of individuals (*n* = replicates) for each species and mean and associated standard deviation of individual mass are given, as well as range (in parentheses). Coefficients (and confidence interval at 95%) of the Standardized Major Axis (SMA) regression model carried out on each species for $\log_{10}$-transformed $NH_4^+$ and SRP *per capita* excretion rates and associated stoichiometry are given with goodness-of-fit statistics ($R^2$). $R^2$ values in bold indicate significant models. Slope values in bold are significantly different from 1.

 

western European rivers and lakes (Kottelat & Freyhof, 2007).

Fish sampling in rivers was carried out using EFKO F.E.G. 1500 (Leutkirch, Germany) electrofishing gear. In the lakes where bank steepness and water depth made electrofishing inefficient, fish were sampled by angling and gillnetting. Gillnets were set for <15 min to prevent excessive stress or injury to fish. In agreement with French law, all of the fish (except bullheads and pumpkinseeds) were released to the water immediately after the experiments. Black bullheads and pumpkinseeds, which are listed as invasive pests in France, were anaesthetised in eugenol-alcohol solution and then killed using phenoxyethanol.

### *Nutrient excretion assessment*

Nutrient excretion rates were estimated in the field following the protocol of Vanni *et al.* (2002). Immediately after capture, fish were put into buckets filled with site water for 2–5 min to recover. They were then placed individually in a plastic bag filled with bottled spring water stored beforehand at river temperature. For each site, we selected spring water that had similar chemical characteristics to water at the site, particularly in terms of conductivity and pH. Using bottled spring water allows the measurement of nutrient excretion by fish in water having low microbial activity which avoids biased measurement, while minimising the potential physiological stress induced by physicochemical parameters different from those of the natural environment of the fish.

The volume of water was adapted to the size of the individual and ranged from 0.5 L for small individuals (e.g. minnow) to 9 L for large fish (e.g. large chub or barbel). Bags were placed in plastic buckets covered and shaded to reduce fish stress. Incubation time ranged from 40 to 75 min following a trade-off between limit of detection of targeted nutrients and animal welfare (Whiles *et al.*, 2009). Moreover, we preferred to minimise the time spent by fish in plastic bags to avoid the effects of pauses in feeding (Whiles *et al.*, 2009). No mortality, hypoxia or visible stress was observed during the experiments. Previous studies had shown that the field assessments of nutrient excretion by fish are consistent with predictions from bioenergetic models (Vanni, 2002; Torres & Vanni, 2007).

After being removed from the bag, the fish were anaesthetised using a eugenol–alcohol solution then measured to the nearest millimetre and weighed to the nearest 0.1 g. The fish were held in a recovery tank before being released. A water sample of 50 mL was taken from the bag, filtered on 0.45-μm Millipore filter (Millipore, Billerica, MA, U.S.A.) and then stored in an electric cooler in the field, then refrigerated in the laboratory. Samples were analysed the next day for ammonium ($NH_4^+$) and soluble reactive phosphorus (SRP) concentration using the phenol-hypochlorite and molybdenum blue methods, respectively (Torres & Vanni, 2007), in an autoanalyser (ALPKEM FS IV+; O.I. Analytical, College Station, TX, U.S.A.).

Every day two control samples were prepared, consisting of 1 L of bottled spring water placed in a plastic bag for 1 h. Chemical analyses confirmed that the final nutrient concentrations in these samples were identical to that in the corresponding bottled spring water, indicating that the plastic bags did not release or absorb nutrients.

*Per capita* excretion rates for $NH_4^+$ and SRP (μmol $ind^{-1}$ $h^{-1}$) were computed for each replicate as follows (Vanni *et al.*, 2002):

$$\mathrm{Excr_I} = \frac{([\mathrm{I}]_{\mathrm{final}} - [\mathrm{I}]_{\mathrm{initial}}) \times \mathrm{vol}}{\mathrm{time}}$$

with vol being the volume (L) of the water in the plastic bag and *time* the time (hours) the individual was held in the bag. $[\mathrm{I}]_{\mathrm{final}}$ is the final concentration of ion *I* in the water (μM) and $[\mathrm{I}]_{\mathrm{initial}}$ the concentration observed for controls.

*Per capita* excretion rates thus correspond to the molar amount of $NH_4^+$ or SRP excreted by one individual per unit time. The N:P molar ratio of excretion rates was also computed for each replicate. These values, as well as body mass, were $\log_{10}$-transformed prior to all statistical analyses (Vanni *et al.*, 2002).

### *Statistical analyses*

The aim of this study was not to build predictive models of excretion rate given fish mass but to assess the parameters of the allometric relationship between fish body mass and nutrient excretion rate, that is, the intercept (*a*) and slope (*b*) of the linear relationship computed on log-transformed variables: $\log_{10}(excretion) = a + b \times \log_{10}(mass)$. Therefore, given this aim, the best line-fitting method is standardised major axis regression (SMA; Warton *et al.*, 2006). The SMA method differs from the ordinary least-squares regression method in the direction the residuals are computed which accounts for the slope of the fitted line (Warton *et al.*, 2006).

The allometric relationship between body mass and the *per capita* $NH_4^+$ and SRP excretion rates, and the N:P stoichiometric ratio, was assessed on all the replicates using

SMA regression. The slope of each of these three allometric relationships was then tested to see whether it differed significantly from 1. Then an SMA model, including species effect, was computed to test species differences in allometric scaling of $NH_4^+$ and SRP excretion rates and N:P stoichiometry. This procedure first fits an allometric relationship for each species and then tests whether all the species share a common slope (Warton *et al.*, 2006). This analysis also provides the estimated parameters of the allometric relationship for each species, which allows testing of whether the slope of each species differs from 1. All these SMA regressions were computed using the R function *sma* from the package *smatr* (Warton *et al.*, 2012).

We tested the phylogenetic signal in mean body mass, mean excretion rate and mean N:P stoichiometric ratio, and slope of the allometric relationship between mass and excretion rate, using the *K* statistic as implemented in the *picante* package (Kembel *et al.*, 2010). This statistical test compares the observed phylogenetic signal in a trait (computed based on the variance–covariance structure observed in the data) to the expected phylogenetic signal under a Brownian motion model of trait evolution (Blomberg, Garland & Ives, 2003). *K* values of 1 correspond to a Brownian motion process; *K* values >1 indicate strong phylogenetic conservatism, whereas *K* values closer to zero correspond to a random or convergent pattern of evolution. The statistical significance of the phylogenetic signal was evaluated by comparing observed patterns to a null model of shuffling species labels across the tips of the phylogeny (Blomberg *et al.*, 2003). The phylogenetic tree we used was extracted from Grenouillet *et al.* (2011).

At the interspecific level, the effect of body mass on excretion rate and stoichiometry was assessed using SMA regression on average values per species. Then a SMA regression accounting for the phylogenetic signal was implemented using the *phyl.rma* function from the *phytools* R package (Revell, 2012). This analysis used the phylogenetic distance between species to set a covariance structure using a Brownian algorithm (Martins & Hansen, 1997).

Differences in *per capita* nutrient excretion rate encompass both differences in body mass and effect of mass on nutrient excretion within each species. Therefore, analysing the ecological consequence of intra- and interspecific variability in nutrient excretion rate cannot be achieved simply by comparing the parameters of the allometric relationship between mass and nutrient excretion. With this aim, for each species, we estimated a biomass-standardised excretion rate (Vanni *et al.*, 2002; Hall *et al.*, 2007). More particularly, we considered three contrasting sizes by computing first, second (i.e. median) and third quartiles on the body mass values observed for all the replicates of each species. Then, for each of these three sizes, *per capita* $NH_4^+$ and SRP excretion rates were estimated based on the allometric relationship if the corresponding SMA regression model was significant. If the SMA model was not significant, we estimated *per capita* excretion rate by multiplying body mass by the average mass-specific excretion rate (i.e. *per capita* excretion rate divided by individual mass) computed for each species (McIntyre *et al.*, 2008). These estimated *per capita* excretion rates were finally multiplied by the appropriate number of individuals required to reach a total biomass of 1 kg.

All the statistical analyses were performed using R (R Development Core Team, 2011).

## Results

### *Inter-individual variability in nutrient excretion rate*

Nutrient excretion was assessed for a total of 18 species and 470 fish with at least five replicates per species (Table 1). Fish body mass ranged from <1 g to more than 1 kg (Table 1).

*Per capita* excretion rate showed a wide range among the 18 species, from 0.2 to 518 μmol N $h^{-1}$ and from 0.03 to 29 μmol P $h^{-1}$. Similarly, the molar ratio of N:P excretion was highly variable, ranging from 0.8 to more than 280, but globally $NH_4^+$ and SRP *per capita* excretion rates were strongly correlated (Fig. 1).

Individual body mass had an overall significant positive effect on both $NH_4^+$ and SRP excretion rates (Fig. 1). The allometric coefficient between mass and $NH_4^+$ excretion rate was higher than that for SRP excretion rate, and both were significantly <1 ($0.95 \pm 0.04$, 95% CI and $0.81 \pm 0.05$, respectively). Body mass had a significant, although weaker, effect on N:P stoichiometric ratio (Fig. 1).

### *Intraspecific versus interspecific variability in nutrient excretion rate*

Standardised major axis regression accounting for species identity showed that the slope of the allometric relationship between body mass and $NH_4^+$ excretion rate differed significantly among species ($P < 0.001$). The same result was obtained for SRP excretion rate and for N:P stoichiometry.

At the intraspecific level, body mass had a significant positive effect on $NH_4^+$ *per capita* excretion rate for 16 of the 18 species, the two exceptions being the pumpkinseed and the common dace (Table 1). Five of these 16 species showed an allometric coefficient significantly <1, white

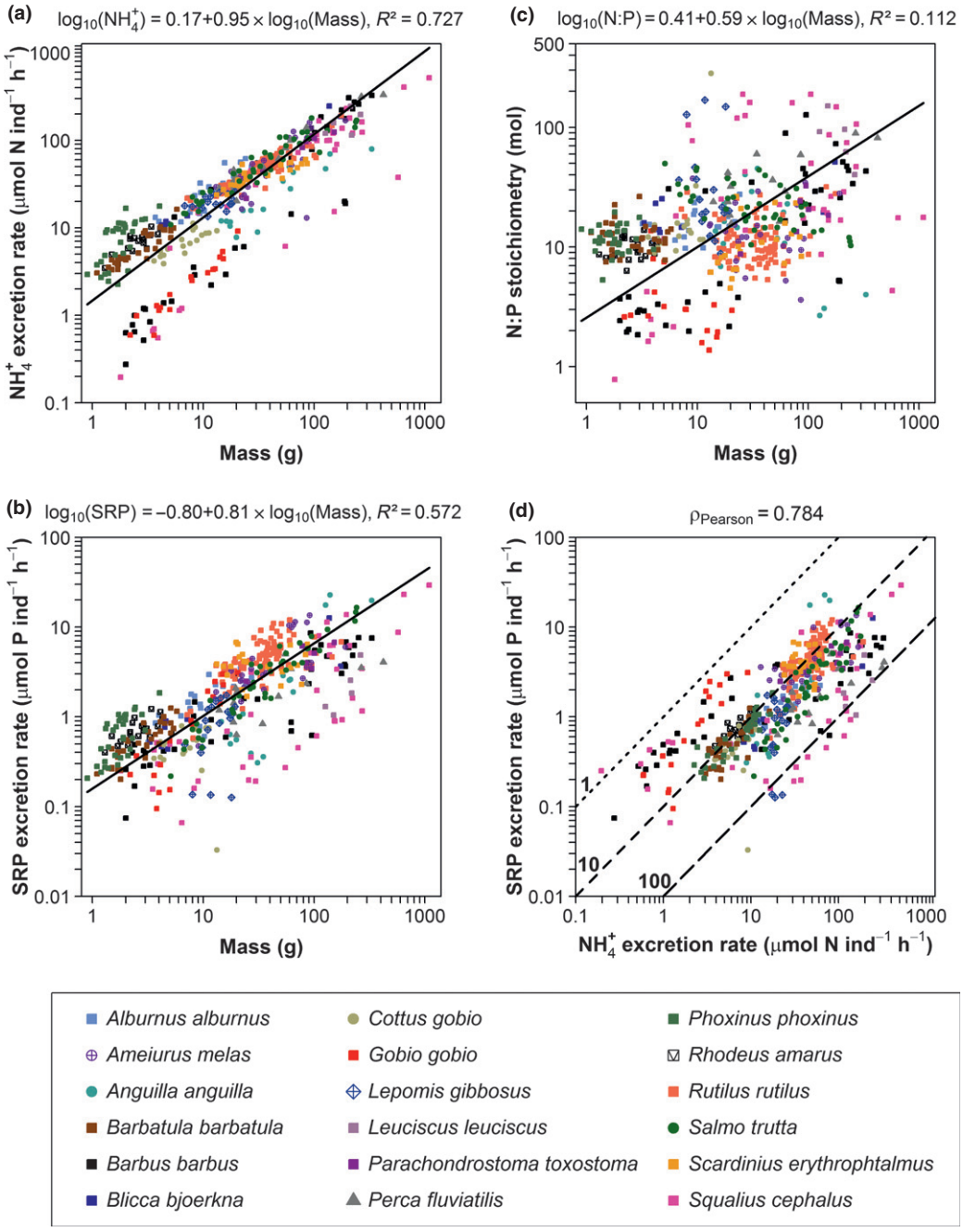


**Fig. 1** Variability in *per capita* excretion rates. Standardised Major Axis (SMA) regression between individual body mass and *per capita* (a) $NH_4^+$ and (b) SRP excretion rates and (c) N:P stoichiometry are drawn with the corresponding equations shown above each panel. Pearson's correlation test between $NH_4^+$ and SRP *per capita* excretion rates is shown above the bottom right panel (d).

bream, toxosotome, roach, brown trout and rudd, while two species showed an allometric coefficient significantly >1, barbel and Eurasian minnow (Table 1).

Similarly, body mass had a significant positive effect on SRP *per capita* excretion rate for 13 species (Table 1). The five species showing no significant relation were again pumpkinseed and dace, together with bullhead, toxostome and rudd (Table 1).

Two species showed an allometric coefficient significantly <1, barbel and European perch, while three species showed an allometric coefficient significantly >1, European eel, gudgeon and Eurasian minnow (Table 1).

Finally, the N:P stoichiometric ratio of excretion rates was affected significantly by body mass in only eight species, five showing an allometric coefficient significantly <1 (European eel, barbel, gudgeon, European perch

and brown trout), while only the bullhead showed an allometric coefficient significantly >1 (Table 1).

The number of replicates used to estimate the allometric relationship between mass and $NH_4^+$ (or SRP) excretion rate for each species was not significantly correlated with the width of the confidence interval of the estimated slope (Pearson's coefficient of correlation $\rho = 0.235$, $P = 0.347$ for $NH_4^+$ excretion, $\rho = 0.278$, $P = 0.265$ for SRP excretion). Similarly, the range of fish body mass used to estimate the allometric relationship was not correlated with the width of the confidence interval of the estimated slope ($\rho = 0.167$, $P = 0.508$ for $NH_4^+$ excretion, $\rho = 0.30$, $P = 0.906$ for SRP excretion).

### *Phylogenetic signal in interspecific variability of nutrient excretion rate*

Mean body mass was very variable with values ranging from 2 g for the Eurasian minnow to *ca* 200 g for the common dace (Fig. 2). There was no phylogenetic conservatism of body mass among the 18 species ($K = 0.460$, $P = 0.109$). Similarly, mean $NH_4^+$ and SRP excretion rates had a 100-fold and 10-fold difference between species, respectively, while the mean N:P molar ratio ranged from 3 to 88. Again, no significant phylogenetic conservatism was found for SRP excretion rate ($K = 0.488$, $P = 0.119$) or N:P stoichiometry ($K = 0.409$, $P = 0.444$). Ammonium excretion rate showed a higher, but still not quite significant, level of phylogenetic conservatism ($K = 0.536$, $P = 0.052$). The slope of the allometric relationship was not significantly conserved among species for $NH_4^+$ ($K = 0.456$, $P = 0.500$) and SRP ($K = 0.607$, $P = 0.320$) excretion rates or for N:P stoichiometry ($K = 0.441$, $P = 0.271$).

Mean $NH_4^+$ and SRP excretion rates computed for each species, as well as the N:P ratio, increased significantly with the mean body mass of the species (Fig. 2). Parameters of the allometric relationship estimated by SMA regression accounting for the phylogenetic distance between species were similar to the SMA model not accounting for the phylogenetic signal for both $NH_4^+$ and SRP excretion (Fig. 2). The goodness-of-fit values ($R^2$) of the two types of SMA models were also very close (Fig. 2). Therefore, considering phylogenetic relatedness among species did not improve the predictive power of body mass on excretion fluxes at the interspecific level.

### *Biomass-standardised nutrient excretion rate*

Biomass-standardised $NH_4^+$ excretion rate varied by a factor of 16, from 279 to 4407 mmol $kg^{-1}$ $h^{-1}$ (Fig. 3) and biomass-standardised SRP excretion rate varied by a factor of 34 from 11 to 375 mmol $kg^{-1}$ $h^{-1}$ (Fig. 3). The rate of excretion of SRP, standardised for biomass, decreased more strongly with increasing body mass than $NH_4^+$ excretion (Fig. 3) and the N:P ratio tended to increase with body mass (from 2.2 to 81). Intraspecific differences in biomass-standardised excretion fluxes were universally lower than interspecific differences, except for species with a large size range (e.g. barbel).

## Discussion

Nutrient excretion rates showed a large variability both within and among the 18 fish species studied. *Per capita* excretion rate varied overall by a factor of 1000 for both N and P, reaching 518 μmol N $h^{-1}$ and 29 μmol P $h^{-1}$. Nitrogen excretion was higher than P excretion for all but one individual and the N:P molar ratio reached more than 250. These values obtained for European freshwater fish are within the range of values observed for five North American lake fish (Torres & Vanni, 2007) and for 39 neotropical riverine fish species (McIntyre *et al.*, 2008).

Nitrogen and P *per capita* excretion rates, as well as the N:P ratio, increased significantly with fish body mass among the 18 species (Fig. 1). Body mass had a stronger effect on N than on P excretion rate, and the slopes of these allometric relationships were both lower than 1 (Fig. 1). Overall, the values of these scaling coefficients confirm that excretion rate per unit mass decreases slightly with fish mass and that the N:P ratio tends to increase with fish mass (Vanni *et al.*, 2002; Sereda *et al.*, 2008a). The slopes found for European fish were very close to those reported for 49 non-detritivorous fish species (Sereda *et al.*, 2008a), that is, 0.95 versus 0.92 for N and 0.81 versus 0.79 for P. However, another meta-analysis on mean excretion rates for 30 fish species found a slope not significantly different from 1 for N and P excretion (Hall *et al.*, 2007). Therefore, it would be necessary to assess excretion rates on more species to reach a better estimation of the allometric relationships between mass and excretion rates.

Furthermore, the strength of the effect of body mass on nutrient excretion rate differed significantly among species, indicating that besides differences in body mass, species identity also influences *per capita* nutrient flux (Tables 1 & 2). These patterns are consistent with works on other fish faunas (Vanni *et al.*, 2002; Hall *et al.*, 2007; McIntyre *et al.*, 2008; Sereda *et al.*, 2008a; Sereda & Hudson, 2011; Small *et al.*, 2011) which also found significant interspecific differences besides the predominant effect of body mass. More particularly, while N and

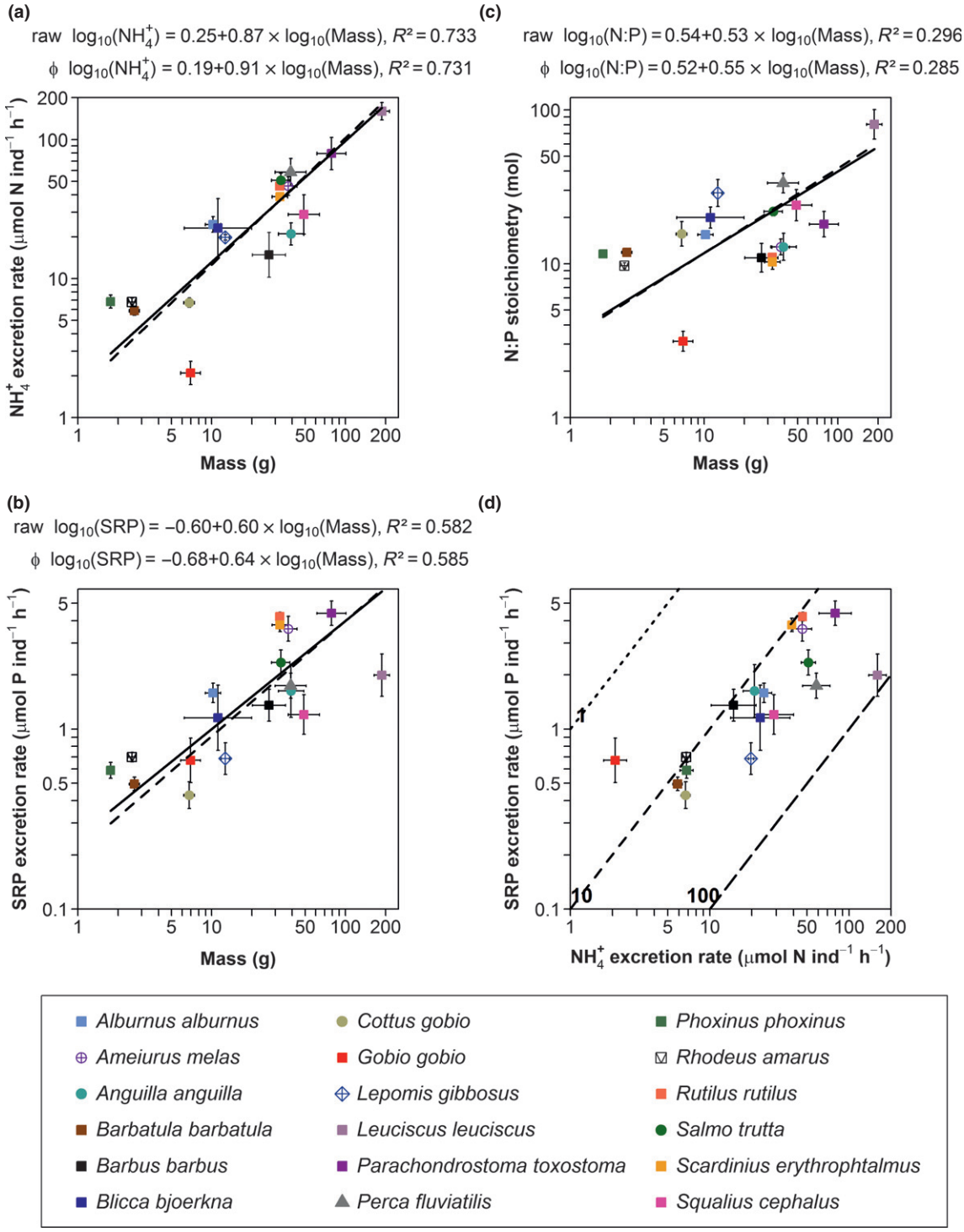


**Fig. 2** Interspecific differences in nutrient excretion rates. Mean values and associated standard errors computed for each species. Effect of body mass on (a) $NH_4^+$ and (b) SRP excretion rates and (c) N:P stoichiometry assessed using Standardised Major Axis (SMA) regression model. Solid and dashed lines represent SMA models accounting ('Φ') or not ('raw') for phylogenetic distances between species, respectively. $NH_4^+$ versus SRP excretion rate is shown in the bottom right panel (d).

P excretion rates increased significantly with individual body mass for most of the European species studied, two and five species showed no significant effect of body mass on N and P excretion rates, respectively (Table 1). This proportion of species is low compared to the 39 Neotropical species studied by McIntyre *et al.* (2008) in which 12 and 27 species showed no significant relationship for N and P, respectively.

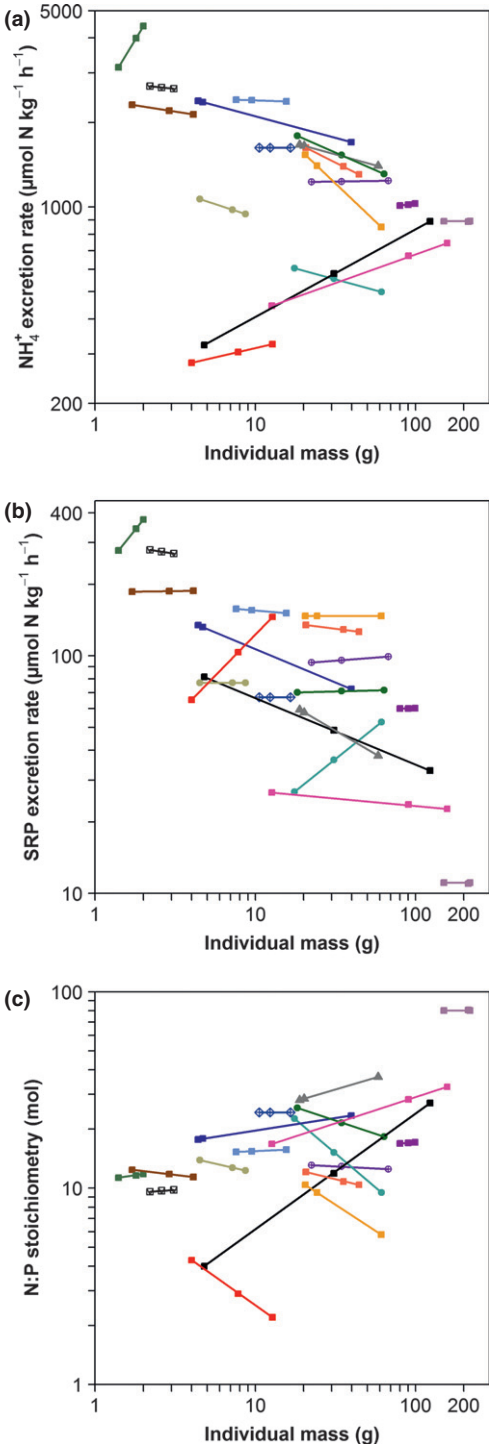


**Fig. 3** Variability in mass-standardised nutrient excretion rates. Molar amount of (a) N and (b) P excreted per hour by 1 kg of each species and (c) N:P stoichiometric ratio, considering three individual body mass values (i.e. first, second [median] and third quartile). Species colour codes are as in Fig. 2.

Only two and three species showed a scaling coefficient (slope of the log–log relationship) significantly >1 for N and P excretion rates, respectively (Table 1). Again, such patterns were also found for Neotropical fish, with six and 10 species having a scaling coefficient higher than 1 for N and P excretion rates, respectively (McIntyre *et al.*, 2008), although it should be noted that these authors used ordinary least-squares regression that underestimates the allometric scaling coefficient (Warton *et al.*, 2006). Further research should thus aim at disentangling the factors influencing interspecific differences in basal excretion rate as well as the effect of body mass on excretion rate. Excretion is only one of the components of the fish nutrient budget, and it is thus influenced by the trade-off between nutrient supply through food intake and nutrient demand for tissue building and maintenance (Vanni, 2002; Vanni *et al.*, 2002; McIntyre & Flecker, 2010). For instance, species of the Loricaridae have body plates that contain a high proportion of P, but feed on P-poor periphyton and have a low P excretion rate (Vanni *et al.*, 2002; Hood, Vanni & Flecker, 2005). This trade-off between nutrient supply and demand on nutrient excretion rates also acts at an intraspecific level, especially following ontogenetic changes in diet and/or morphology. For instance, a shift from a zooplanktonivorous to a detritivorous diet can produce marked changes in nutrient excretion rate and stoichiometry (Pilati & Vanni, 2007; Sereda *et al.*, 2008a).

Our results showed that the absence of a significant allometric relationship between fish body mass and excretion rate was not because of statistical constraints, such as low size range and/or low number of individuals sampled. Therefore, it would be challenging to test whether the absence of variation of excretion rate with fish mass observed for some species results from ontogenetic shifts in diet and/or body composition, which can blur the different allometric relationships of excretion rates within juvenile and adult stages. Similarly, it is necessary to understand why some species show a scaling coefficient higher than 1 and particularly whether these extreme allometric relationships are driven by ontogenetic changes (e.g. from non-detritivory to detritivory; Sereda *et al.*, 2008a). In addition, the scaling coefficients for N and P excretion generally differ within species, which results in changing N:P ratios with fish mass. For most species, the scaling coefficient of N excretion is higher than that of P, and consequently the N:P ratio in excretion fluxes increases with body mass. However, some species show the opposite trend and it remains a challenge to understand why.

Investigations along these lines will require extensive data on diet, ingestion rate, fish growth and body nutrient content at the individual level to disentangle the relative contributions of each of these drivers (Vanni *et al.*, 2002;

Glaholt & Vanni, 2005; Pilati & Vanni, 2007; Verant *et al.*, 2007; Sereda & Hudson, 2011; Small *et al.*, 2011). Here, even though we were not able to measure body nutrient concentration, most of the fish were cyprinids and may thus have similar body nutrient content (Hendrixson *et al.*, 2007). Similarly, among the 18 fish species studied, there were no strictly detritivorous fish and only one herbivore (toxosotome that grazes on periphyton). This last species did not exhibit marked differences in its nutrient excretion compared to the other species which were mostly invertivorous, although some can also feed on fish (e.g. European perch). In contrast, species sharing a similar diet can have distinct excretion rates. For example, the gudgeon had a N excretion rate three times lower than the bullhead but a P excretion rate 1.5 times higher (Fig. 2), while both species feed on benthic invertebrates.

The interspecific differences found in average nutrient excretion rate among the 18 species were also influenced by fish mass, although the slopes of these allometric relationships were lower than slopes estimated on individual excretion rates (0.95 versus 0.87 and 0.81 versus 0.60 for N and P, respectively; Figs 1 & 2). Moreover, these differences were not influenced by phylogenetic relatedness among species. This finding could be viewed with respect to the fact that body mass was not phylogenetically conserved (Fig. S1), while it has a strong effect on nutrient excretion. These first evaluations of phylogenetic constraints on nutrient excretion and on the scaling of allometric relationships may not be true for more diversified fish faunas (e.g. considering diet or morphology), as in the tropics (Vanni *et al.*, 2002; McIntyre *et al.*, 2007; Small *et al.*, 2011). For instance, previous studies reported a significant phylogenetic signal in body P concentration among North American fish species (Hendrixson *et al.*, 2007). It would thus be challenging to test whether body nutrient content and nutrient excretion rate show phylogenetic conservatism based on a larger species pool, including temperate and tropical clades. If no phylogenetic signal is found, it would suggest that phylogenetically close species can have contrasting excretion rates and, thus, that taking phylogeny into account will not improve the predictive performance of allometric models.

The large intra- and interspecific variability in *per capita* nutrient excretion rate, revealed here, coupled to differences in fish mass, led to marked differences in nutrient recycling at the population level (i.e. biomass-standardised excretion rate). First, following the general allometric relationship between mass and excretion rate, small individuals tend to excrete more nutrient per unit mass than bigger ones (Fig. 3). For example, the number of moles of $NH_4^+$ excreted by 1 kg of Eurasian minnow, with an individual mass of 1.8 g, is almost 4 mmol $h^{-1}$, which is six times greater than the amount excreted by 1 kg of chub (individual body mass of 90 g). The difference between these two species even reached 14-fold for $PO_4^{3-}$ excretion (Fig. 3). The discrepancy between the effect of mass on N and P also led to an N:P ratio 2.4 times greater for chub than Eurasian minnow.

Moreover, given the interspecific variability in allometric relationships between mass and excretion rate, marked divergences were also observed between species with fairly similar individual body mass. For example, 1 kg of gudgeon (individual mass 7.8 g) excreted eight times less $NH_4^+$ but 1.5 times more $PO_4^{3-}$ per unit time, than 1 kg of small bleak (individual mass 7.6 g). Furthermore, whereas for most fish species, biomass-standardised excretion rate tends to decrease with increasing fish mass (i.e. the slope of the allometric relationship is lower than 1), the magnitude of this change varies among species following differences in the scaling coefficient of the allometric relationship between body mass and excretion rates (Table 1). More importantly, few species showed an increasing biomass-standardised excretion rate with fish mass (i.e. the slope of the allometric relationship is >1). For instance, a group of small barbel of 5 g each excreted three times less N per unit time than the same total biomass of 123-g barbel (scaling coefficient of 1.31). In contrast, the group of large barbel excreted 2.5 times less P than the small fish, because of the scaling coefficient of 0.72 for P excretion. Hence, the molar ratio of excretion was only four for small individuals and more than 27 for large ones (Fig. 3).

Nutrient recycling at the ecosystem level is influenced by both the fish community structure (i.e. species composition, biomass and size structure) and the nutrient excretion rates of these species (Hall *et al.*, 2007; McIntyre *et al.*, 2008). Therefore, the intra- and interspecific variability in *per capita* excretion rate found in this study for the dominant European fish species is the first step towards a better assessment of contribution of fish to nutrient cycling in European freshwater ecosystems. All these potential effects of diversity in fish nutrient excretion rates and community structure on nutrient recycling need to be experimentally tested using *in situ* (Taylor, Flecker & Hall, 2006; Schaus *et al.*, 2010) or mesocosm experiments (Kohler *et al.*, 2011; Mette *et al.*, 2011), or modelling approaches (Tarvainen *et al.*, 2002; McIntyre *et al.*, 2007). Future studies will have to address in particular the synergistic effects of changes in the structure of fish communities (species composition and size distribution) and the abiotic changes in water temperature

and nutrient availability on ecosystem processes and stability.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Phylogenetic conservatism of body mass, nutrient excretion rates and N:P stoichiometry of 18 fish species.

**Table S1.** List of species studied.

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