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Organismal stoichiometry at the temporal scale: Seasonal variability shapes interspecific differences in fish

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Abstract

1. Changes in organismal stoichiometry (OS) may be driven by seasonal changes in lipid reserves (i.e. energy) and gonadal development in fish. However, these relationships are understudied. Hence, we investigated how seasonal changes in body lipid content and gonadal development can drive the seasonal variability of OS traits at the example of three coexisting freshwater fish species. Furthermore, we aimed to assess the importance of seasonal OS alteration in comparison with interspecific differences.
2. Carbon (%C), nitrogen (%N) and phosphorus (%P) contents, and the molar ratios of these elements (C:N, C:P and N:P) were examined in rudd (*Scardinius erythrophthalmus*), pumpkinseed (*Lepomis gibbosus*) and Amur sleeper (*Perccottus glenii*). We consider the sex and seasonal changes in body size as a potential factor in shaping OS.
3. Substantial seasonal intraspecific variability occurred in all OS traits. Its extent exceeded interspecific differences in %C, %N and C:N, while %P and C:P, N:P were determined primarily by species identity. The effect of sex occurred sporadically and was considerable in some cases. Seasonal changes in total length did not contribute to intraspecific variability of OS. Intra-annual changes in body lipid content affected seasonal variability of OS traits substantially, but the strength of this effect was species-specific. The regulatory role of lipid reserve alterations worked only for those species that exhibited considerable seasonal variation in body fat content. Gonado-somatic index proved to be marginal in shaping seasonal changes in OS, presumably because a substantial portion of the essential elemental demand for gonadal growth is supplied by rearrangements within the body, without notable changes in the entire elemental composition.
4. In the light of our findings, we suggest that more attention should be given to the influence of seasonal variability in OS traits, and sex should be considered as a taxon-dependent effect. Ignoring this substantial degree of variability might lead to inaccuracies in assessing the extent of both intra- and interspecific OS differences. We conclude that seasonal OS variability might shape consumer-driven nutrient dynamics.

KEYWORDS

carbon, elemental composition, lipid content, nitrogen, phosphorus

1 | INTRODUCTION

Over recent decades, the key role of consumers in nutrient cycling in aquatic ecosystems has been increasingly recognised (Capps, Atkinson, & Rugenski, 2015). Studies on the consumer-driven nutrient dynamics frequently focused on the role of fish (Atkinson, Capps, Rugenski, & Vanni, 2017), because, due to the high nutrient (i.e. phosphorus, nitrogen; hereafter P and N) content of their bodies, they constitute biogeochemical hotspots (Kitchell, Koonce, & Tennis, 1975; McIntyre & Flecker, 2010; McIntyre et al., 2008). Fish affect internal nutrient dynamics in numerous ways (see Breukelaar, Lammens, Klein Breteler, & Tátrai, 1994; Horppila, Peltonen, Malinen, Luokkanen, & Kairesalo, 1998; Vanni, 2002; Vanni, Boros, & McIntyre, 2013), and they can act both as sinks and sources of nutrients (Sereda, Hudson, Taylor, & Demers, 2008; Vanni et al., 2013). Relevant studies have emphasised the substantial importance of excretion (Vanni, 2002; Vanni & McIntyre, 2016; Wheeler, Miller, & Crowl, 2015) as the most direct effect. In fact, nutrient excretion by fish can support the entire primary production demand for P in nutrient-poor aquatic ecosystems (Small, Pringle, Pyron, & Duff, 2011; Vanni, Flecker, Hood, & Headworth, 2002).

Excretion patterns of consumers (i.e. rates and ratios of nutrient recycling) are strongly regulated by mismatches between nutritional demand (body elemental composition) and supply (food elemental composition; Schindler & Eby, 1997). Ecological stoichiometry (ES) theory (Elser & Urabe, 1999; Sterner, 1990; Sterner & Elser, 2002) provides a mechanistic framework for the relationship between demand and supply. ES theory applies the elemental composition and elemental ratios in a consumer's body (hereafter organismal stoichiometry; OS) as a proxy for nutritional demand and assumes that heterotrophs, particularly vertebrates, maintain species-specific and relatively constant OS in the face of the nutrient imbalances between their body and ingested food (Vanni et al., 2002). Consequently, investigations on OS are essential for the better understanding of consumer-driven nutrient dynamics.

Since one of the central concepts of ES theory was that heterotrophic species maintain relatively constant elemental composition, most studies on OS of fish have focused on describing interspecific differences (e.g. Sterner & George, 2000; Vanni et al., 2002). These studies reported a high degree of interspecific variability and revealed strict linkage between OS traits and phylogeny of species (Dantas & Attayde, 2007; Hendrixson, Sterner, & Kay, 2007). However, subsequent studies reported unexpectedly high intraspecific OS variability in fish (e.g. Pilati & Vanni, 2007; Vrede et al., 2011). Accordingly, the number of investigations on intraspecific OS variability has substantially increased (e.g. Dickman, Newell, González, & Vanni, 2008; Ebel, Leroux, Robertson, & Dempson, 2015; El-Sabaawi, Kohler et al., 2012; El-Sabaawi, Travis et al., 2012). Recent studies have revealed that environmental heterogeneity can lead to considerable OS differences among different populations of a single species (Boros et al., 2012; de Andrade Santos, de Freitas Terra, Zandoná, Santaella, & Rezende, 2016; Leal, Best, Durston, El-Sabaawi, & Matthews, 2017).

The within-year changes in environmental conditions induce considerable seasonal fluctuations in the lipid (L) content of fish bodies (Brown & Murphy, 2004; Jonas, Kraft, & Margenau, 1996; Konečná & Reichard, 2011). For instance, in temperate climate, the food limitation during winter reduces L reserves (Copeland, Murphy, & Ney, 2010; Hurst & Conover, 2003; Shuter, Finstad, Helland, Zweimüller, & Hölker, 2012), while the increase in available food resource and the high L (i.e. energy) requirements of gonadal development (Johnson, 2009; McBride et al., 2015) lead to increase the L content of body in spring and summer. Because L constitutes the most important carbon (C) storage pool in fish bodies (Czamanski et al., 2011; Fagan, Koops, Arts, & Power, 2011), it can be hypothesised that within-year changes in this pool would result in seasonal variation of OS. Along with this process, intense protein synthesis in the gonads increases N and P demand (Dawson & Grimm, 1980; Hendry, Dittmen, & Hardy, 2000; Shearer, 1984). This is because N is an essential component of amino acid and protein synthesis, and because a large quantity of P-rich ribonucleic acid is required (Elser et al., 2003; Vrede, Dobberfuhl, Kooijman, & Elser, 2004). Therefore, changing in nutritional demand during the annual reproductive cycle may reasonably affect OS of fish.

To explore the potential within-year variability in the OS of fish, we measured elemental composition (%C, %N and %P), molar ratios of these elements (C:N, C:P and N:P) and L content (%L) in samples of three freshwater fish species collected at different seasons, and characterised gonadal development of individuals. Our specific predictions were as follows: (1) %L would change substantially between seasons; (2) significant seasonal differences in C-related OS traits (i.e. %C, C:N and C:P) would also occur, driven mainly by seasonal alterations of %L; (3) %N, %P and N:P would vary moderately among seasons; (4) the effect of %L on these OS traits either would be marginal or would be exerted via stoichiometric dilution; and (5) gonadal development (expressed as gonado-somatic index, hereafter GSI) would exert a significant effect on each OS trait and would lead to sex-specific differences. Furthermore, we aimed to assess the relative influence of seasonal, intraspecific variability of OS in comparison with interspecific differences. Hence, different fish species were sampled simultaneously from a wide taxonomic range, which allowed us to compare directly the relative contribution of seasonality (i.e. intraspecific variability among seasons) with the species identity (i.e. interspecific differences) in shaping OS.

2 | METHODS

2.1 | Study site, sampling and study species

Fish were sampled from a eutrophic oxbow lake connected to the north-eastern section of the River Tisza in Hungary (48°05'N, 21°27'E), during spring (April), summer (July) and autumn (October) of 2012. The oxbow has a surface area of 0.9 km² and a mean depth of 1.8 m. Sampled fish species were rudd (*Scardinius erythrophthalmus*, Cyprinidae), pumpkinseed (*Lepomis gibbosus*, Centrarchidae) and Amur sleeper (*Perccottus glenii*, Odontobutidae). Altogether, 192

individuals were captured for the study (see Supporting information Table S1 for more details). The collection was approved by the Government Office for Hajdú-Bihar County, Hungary (permission number: IX-H-001/2767-3/2012).

Rudd is an indigenous herbivorous/omnivorous cyprinid species. Pumpkinseed is native to North America, while Amur sleeper originates from Far-East Asia. The spawning periods of the three species overlap and occur between April and July, when the water temperature is above 15°C. Pumpkinseed and Amur sleeper are multiply-spawning fish species, and parental care is provided by the male. All study species prefer nutrient-rich, well-vegetated lowland rivers, lakes and oxbows (Kottelat & Freyhof, 2007).

2.2 | Sample preparation and analyses

Standard length (mm), total length (mm) and wet mass (g) of the captured fish were recorded. Fish were euthanised by immersion in ice slurry (Blessing, Marshall, & Balcombe, 2010) and dissected to identify sex, weigh gonads and remove gut contents. Gonads and empty guts were then placed back into the fish. GSI was calculated for each individual as follows:

$$\text{GSI} = \text{wet total body mass (g)} / \text{wet mass of gonad (g)} \times 100. \quad (1)$$

Carcasses were dried to constant weight at 60°C and were ground to a fine powder with a Retsch ZM 200 ultracentrifugal mill (Retsch GmbH, Haan, Germany).

Subsamples of the homogenates were analysed for whole body %C and %N in two or three replicates using a Vario EL CNS analyser (Elementar Analysensysteme GmbH, Langenselbold, Germany). For P content analysis, subsamples were incinerated at 550°C for 8 hr and the produced ash was dissolved in 0.3 N HCl solution at 105°C for 1 h (Boros & Mozsár, 2015). Three or four replicates per each sample were run. The liberated orthophosphate ions were quantified with a standard colorimetric molybdenum-blue method. The L content of subsamples was extracted overnight with a 2:1 mixture of chloroform-methanol at 20°C in two or three replicates (Brown & Murphy, 2004; Folch, Lees, & Sloane-Stanley, 1957). After centrifuging the suspension, the solvent was evaporated and the residual L was measured gravimetrically. The elemental contents (%C, %N and %P) as well as the %L were expressed as percentage of dry mass, while ratios of elements (C:N, C:P and N:P) were expressed in molar units.

2.3 | Data analyses

Before the main statistical analyses, we used three-way ANOVAs (type III SSQ) in a crossed factorial design to assess the dependency of each covariate (%L, GSI and total length [TL]) on the factors (season, species and sex). These preliminary tests were necessary because using both covariates and factors as explanatory variables in linear models (ANCOVA) require independency between the covariates and the factors (Quinn & Keough, 2002).

Preliminary ANOVA tests showed significant effects of the factors on all covariate variables (Supporting information Table S2);

therefore, we excluded the covariates from the subsequent main statistical analyses. Therefore, the effects of seasonal changes in covariates on the seasonal variability of OS traits were assessed by comparing the seasonal patterns of covariates and OS traits. To continue, we again fitted three-way ANOVAs (type III SSQ) to each response variable (%C, %N, %P, C:N, C:P and N:P) using the factors (season, species and sex) as explanatory variables in a fully crossed factorial design. After model fitting, two-directional (i.e. both forward and backward) stepwise model selection processes were applied to arrive at the most parsimonious statistical models for the given data.

Except the independence of the sampling units, which was met by random sampling, assumptions of the ANOVA models were checked after model fitting (*a posteriori*) via residual analysis using diagnostic plots (Quinn & Keough, 2002). The models fitted on the data for all response variables with the exception of GSI, where heteroscedasticity and normality were violated. To improve model fitting, we arcsine square root-transformed the GSI data, deleted three outliers and refitted the model.

To assess the relative explanatory power of the terms of the most parsimonious models, partial η^2 values were computed (Field, Miles, & Field, 2012). A partial η^2 value of a given term expresses the portion of variance explained solely by the term in the variance not explained by all the other terms of the model; hence, it is a measure of effectiveness of the terms.

Pairwise comparisons were based on the examination of the 95% confidence intervals of the fitted values (i.e. expected values of a given response variable in each treatment group) of the models (Supporting information Table S3). Differences were considered statistically significant if the confidence intervals of treatment groups did not overlap. Pairwise comparisons were made on the significant highest-way interactions.

All statistical analyses were performed in the R environment (R Core Team, 2013) using the 'multcomp' (Hothorn, Bretz, & Westfall, 2008), 'car' (Fox & Weisberg, 2011), 'effects' (Fox, 2003) and 'lattice' (Sarkar, 2008) packages.

3 | RESULTS

Seasonal variation in %L was taxon-specific (Figure 1a). Summer peaking of %L was found in rudd, while %L declined from spring to autumn in Amur sleeper, and notable seasonal difference was not observed in pumpkinseed. GSI peaked in summer in pumpkinseed and declined in Amur sleeper from spring to autumn (Figure 1b). GSI was the lowest in rudd in summer, but values were relatively low in all seasons. Total length increased significantly from spring to autumn in rudd and showed moderate increase in summer and a slight decline in autumn in Amur sleeper (Figure 1c; Supporting information Table S3). Pumpkinseed TL did not differ notably among seasons.

Species identity and species \times season interactions accounted for the vast majority of variance in each OS trait. In the models for %C, %N and C:N, the species \times season interaction was the

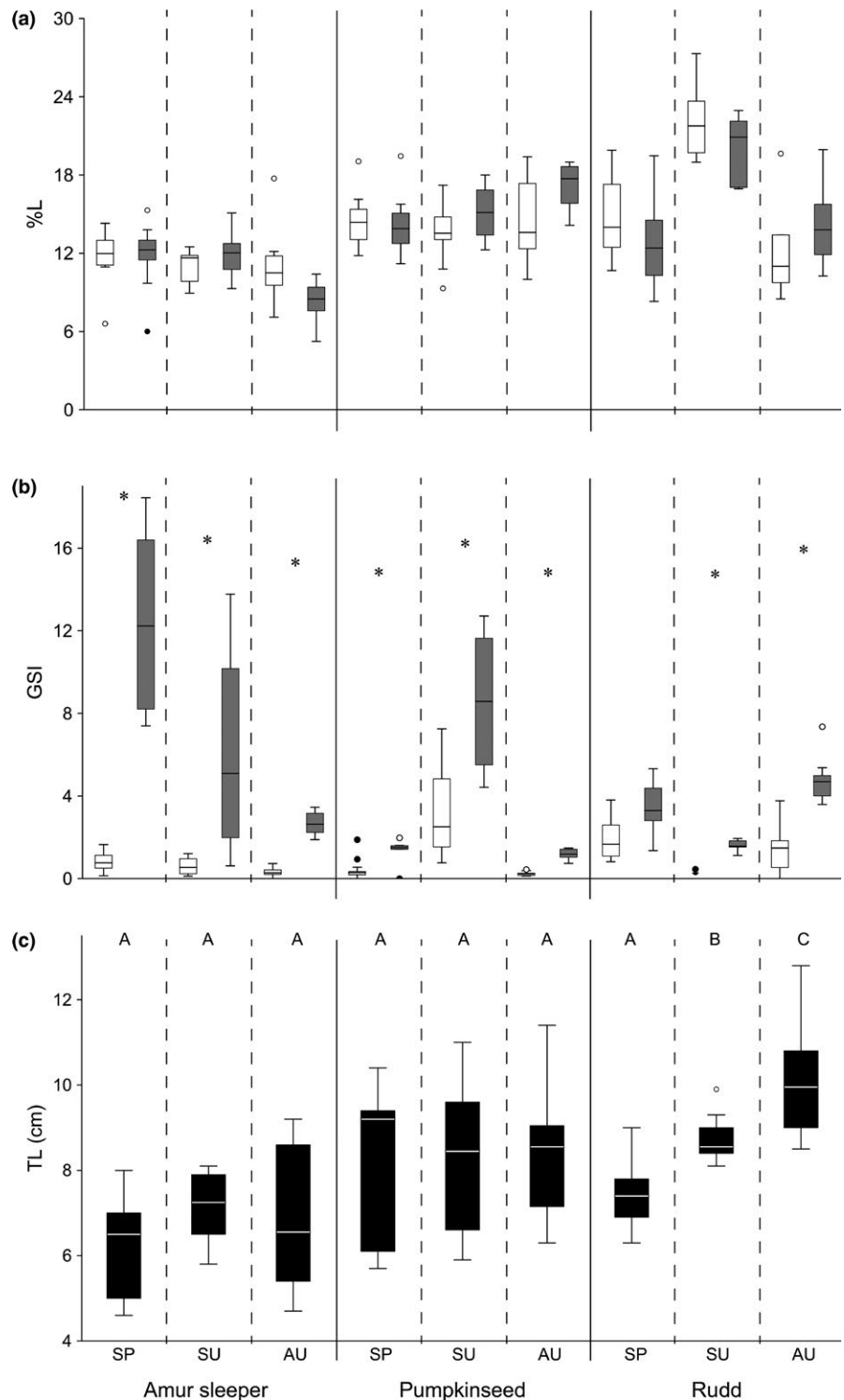


FIGURE 1 Box-plots of (a) lipid content (%L), (b) gonado-somatic index (GSI) and (c) total length (TL). Each box represents the 25% and 75% quartiles, and the line in the box represents the median. The whiskers show the highest and the lowest, non-outlier values, while the open circles and filled circles denote, respectively, the outliers and extremes. Open boxes represent males, grey boxes represent females, and black boxes represent both sexes combined. The asterisk denotes the significant differences between sexes, while the different letters mark the significant differences among seasons. Pairwise comparisons were based on the examination of the 95% confidence intervals of the fitted values (i.e. expected values of a given response variable in each treatment group) of the three-way ANOVA models. In the case of GSI, the pairwise comparisons were made on arcsine square root-transformed data. Comparisons were made between the levels of the highest-way interaction term (treatment group) of the models. SP, spring; SU, summer; AU, autumn

strongest predictor (Table 1), indicating that the seasonal variability was not consistent among species and overwhelmed interspecific differences (see Figure 2). Species \times season interaction was significant in the models for %P and C:P, and N:P, but these OS traits were primarily determined by species identity (Table 1). The explanatory power of the season factor was almost as high as species identity in %C and %N (Table 1). The species \times sex and the species \times season \times sex interactions frequently accounted for a considerable

part of variance, suggesting that OS differences between sexes varied among species and sex affect the species' OS response to seasonality.

The most pronounced interspecific variation occurred in P-related OS traits (i.e. %P, C:P and N:P). The pumpkinseed had the highest mean %P and the lowest mean C:P and N:P, while the lowest mean %P and highest C:P and N:P were observed in Amur sleeper. The interspecific variability was one order of magnitude larger in

P-related OS traits (mean coefficient of variation (CV) = 17.52% for %P, CV = 19.70% for the C:P and CV = 18.15% for N:P) than was documented in the traits associated with C and N (CV = 1.61% for %C, CV = 1.73% for %N, CV = 1.59% for C:N). The extent of seasonal differences in OS traits differed substantially among elements (CV

ranging from 3.20% to 8.49%). The broadest mean seasonal variation was observed in C:P (CV = 8.49%) and %P (CV = 5.56%). Finally, female pumpkinseeds had higher %C, C:N and C:P than males in summer and autumn and female Amur sleepers had higher %C than males in spring (Figure 2a, b).

TABLE 1 Results of three-way ANOVA for organismal stoichiometric traits

Parameter	Terms						Model		
		SS	df	F	p	η^2	F	p	R ² _{adj}
%C	Intercept	260,524	1	1.92×10^5	<0.001	0.999	15.8	<0.001	0.568
	Season	51	2	18.9	<0.001	0.178			
	Species	42	2	15.5	<0.001	0.151			
	Sex	12	1	8.8	0.003	0.048			
	Season × species	169	4	31.1	<0.001	0.417			
	Season × sex	2	2	0.7	0.516	0.008			
	Species × sex	26	2	9.6	<0.001	0.099			
	Season × species × sex	25	4	4.7	0.001	0.097			
	Residuals	236	174			0.500			
%N	Intercept	20,729	1	314,133.4	<0.001	0.999	39.5	<0.001	0.617
	Season	3	2	20.6	<0.001	0.184			
	Species	5	2	36.1	<0.001	0.283			
	Season × species	13	4	50.8	<0.001	0.526			
	Residuals	12	183			0.500			
%P	Intercept	2,026	1	20,839.9	<0.001	0.992	35.2	<0.001	0.753
	Season	0	2	1.4	0.263	0.015			
	Species	39	2	199.1	<0.001	0.696			
	Sex	0	1	2.5	0.117	0.014			
	Season × species	3	4	8.6	<0.001	0.166			
	Season × sex	0	2	1.6	0.202	0.018			
	Species × sex	2	2	8.4	<0.001	0.089			
	Season × species × sex	1	4	3.2	0.014	0.069			
	Residuals	17	174			0.500			
C:N	Intercept	3258	1	1.45×10^5	<0.001	0.999	23.9	<0.001	0.670
	Season	0	2	4.2	0.016	0.046			
	Species	0	2	7	0.001	0.075			
	Sex	0	1	6.5	0.011	0.036			
	Season × species	7	4	75.2	<0.001	0.634			
	Season × sex	0	2	1.3	0.269	0.015			
	Species × sex	0	2	8.5	<0.001	0.089			
	Season × species × sex	0	4	2.5	0.048	0.053			
	Residuals	4	174			0.500			
C:P	Intercept	152,698	1	14,405.9	<0.001	0.988	32.4	<0.001	0.737
	Season	115	2	5.4	0.005	0.059			
	Species	3632	2	171.3	<0.001	0.663			
	Sex	30	1	2.8	0.097	0.016			
	Season × species	712	4	16.8	<0.001	0.278			
	Season × sex	4	2	0.2	0.824	0.002			
	Species × sex	140	2	6.6	0.002	0.070			
	Season × species × sex	178	4	4.2	0.003	0.088			
	Residuals	1,844	174			0.500			

(Continues)

TABLE 1 (Continued)

Parameter	Terms						Model		
		SS	df	F	p	η^2	F	p	R ² _{adj}
N:P	Intercept	7,758	1	18,355.5	<0.001	0.991	31.2	<0.001	0.729
	Season	5	2	5.9	0.003	0.063			
	Species	164	2	194.1	<0.001	0.690			
	Sex	1	1	1.2	0.277	0.007			
	Season × species	9	4	5.5	<0.001	0.113			
	Season × sex	0	2	0.1	0.859	0.002			
	Species × sex	3	2	4	0.019	0.044			
	Season × species × sex	5	4	3.2	0.014	0.069			
	Residuals	74	174			0.500			

Note. %C, carbon content; %N, nitrogen content; %P, phosphorus content; C:N, carbon-to-nitrogen molar ratio; C:P, carbon-to-phosphorus molar ratio; N:P, nitrogen-to-phosphorus molar ratio; η^2 (partial eta squared value), the relative explanatory power of the terms.

A similar pattern was observed in seasonal changes in %L and C-related OS traits (i.e. %C, C:N and C:P) in rudd (see Figures 1a and 2a, b). In Amur sleeper, %C declined along the year similarly to GSI. Similar patterns occurred in changes of GSI and %N, %P in the rudd (compare Figures 1b, 2a). We did not observe similarity in seasonal variation of TL and OS traits.

4 | DISCUSSION

Recent studies on OS of fish revealed the substantial effect of environmental conditions on the elemental composition of body and investigated intraspecific variability primarily at the spatial scale (e.g. El-Sabaawi, Kohler et al., 2012; El-Sabaawi, Travis et al., 2012). However, environmental conditions can undergo substantial intra-annual changes; hence, the investigation of intraspecific variability can be relevant also at the temporal scale. By characterising the intra-annual variability of OS in three coexisting freshwater fish species of different families, we demonstrated significant seasonal variability in OS traits. Moreover, the extent of seasonal variability could exceed the interspecific differences, highlighting the potential importance of the temporal scale in ecological stoichiometric studies.

The seasonal patterns of %L fluctuation and the within-year variability of OS traits in rudd supported our hypothesis: the seasonal changes of L content can drive intraspecific variability of C-related OS traits. Furthermore, considerable L-derived increment in C content can 'dilute' the N and P content of fish bodies (Boros, Sály, & Vanni, 2015; Durston & El-Sabaawi, 2017; Pilati & Vanni, 2007) and can lead to intraspecific variability. However, we did not observe similarities in the seasonal alterations in %L and C-related OS traits in Amur sleeper and pumpkinseed, suggesting that our hypothesis was not generally valid for all fish species. The species-specific differences presumably arose from the widely different %L ranges in the fish species studied. It is also possible that carbohydrates play important role in energy storage in both pumpkinseed and Amur sleeper. Because carbohydrate reserves change more rapidly in response to starvation than L (Love, 1970), this can reduce L content variability and weaken the relationship between L content and C-related OS traits. It has to be noted that

due to the unfavourable weather conditions, we have missed winter sampling and probably the lowest %L values. We speculate that further depletion of L reserves in winter may result in more pronounced seasonal variability in OS.

In contrast to our expectations, the effect of within-year GSI changes on the seasonally variable OS traits was taxon-specific and affected only %C. Given that the demand of gonads for C, N and P is increased during gonadal development (Dawson & Grimm, 1980; Hendry et al., 2000; Shearer, 1984), the experienced species- and OS trait-specific effect was unexpected. One possible explanation is that the nutrient demand during gonadal growth is partially supplied by redistribution of elements within the body rather than additional uptake (Eliassen & Vahl, 1982; Hendry et al., 2000; Medford & Mackay, 1978). This endogenous resource realignment may support the gonadal growth and increase GSI without any changes in the entire elemental composition of the body. In multiple-spawning species such as pumpkinseed and the Amur sleeper, the role of diet-based, exogenous elemental resources can be important in supporting gonadal development (Galloway & Munkittrick, 2006; Johnson, 2009; McBride et al., 2015) and may alter the entire elemental composition. Nevertheless, the contribution of endogenous resources was probably more pronounced in the species involved in this study and thus GSI probably responded to internal element relocation rather than to the accumulation of additional elements. Accordingly, GSI proved to be an inadequate proxy to explore the linkage between reproductive investment and OS traits. Furthermore, the low GSI values found in rudd indicated that our sampling probably missed the period of intense gonadal growth, which may explain the negligible role of GSI in shaping OS. In further studies, a finer temporal scale should be used and the focus should be extended from the simple gonadal development to the whole reproductive investment.

The high L demand of gonadal development suggests a sex-specific variability in C content and C-related OS traits. However, our results failed to support this assumption. For instance, female pumpkinseed exhibited the highest mean %C in autumn, when the reproductive period was over. Furthermore, significant %C differences between sexes occurred in summer samples of

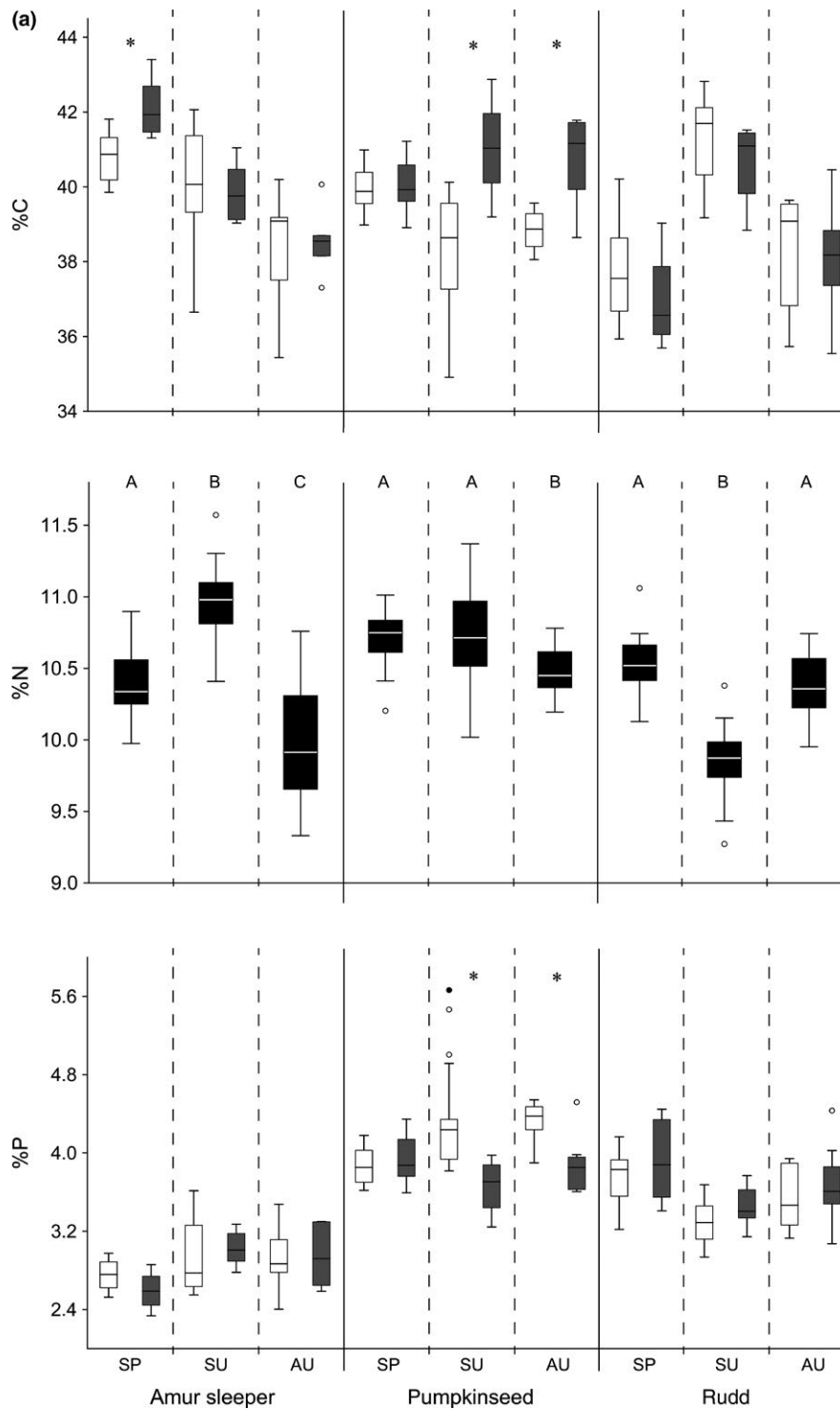


FIGURE 2 Box-plots of organismal stoichiometric traits (a) carbon—%C, nitrogen—%N, phosphorus content—%P and (b) carbon-to-nitrogen—C:N, carbon-to-phosphorus—C:P and nitrogen-to-phosphorus—N:P molar ratios. Each box represents the 25% and 75% quartiles, and the line in the box represents the median. The whiskers show the highest and the lowest values, while the open circles and filled circles denote, respectively, the outliers and extremes. Open boxes represent males, grey boxes represent females, and black boxes represent both sexes combined. The asterisk denotes the significant differences between sexes, while the different letters mark the significant differences among seasons. Pairwise comparisons were based on the examination of the 95% confidence intervals of the fitted values (i.e. expected values of a given response variable in each treatment group) of the three-way ANOVA models. Comparisons were made between the levels of the highest-way interaction term (treatment group) of the models. SP, spring; SU, summer; AU, autumn

pumpkinseed and spring samples of Amur sleeper, when %L did not differ between sexes. On one hand, the males of both species

guard the nest alone, and during this activity, they frequently face food deprivation and substantial reduction in energy reserves

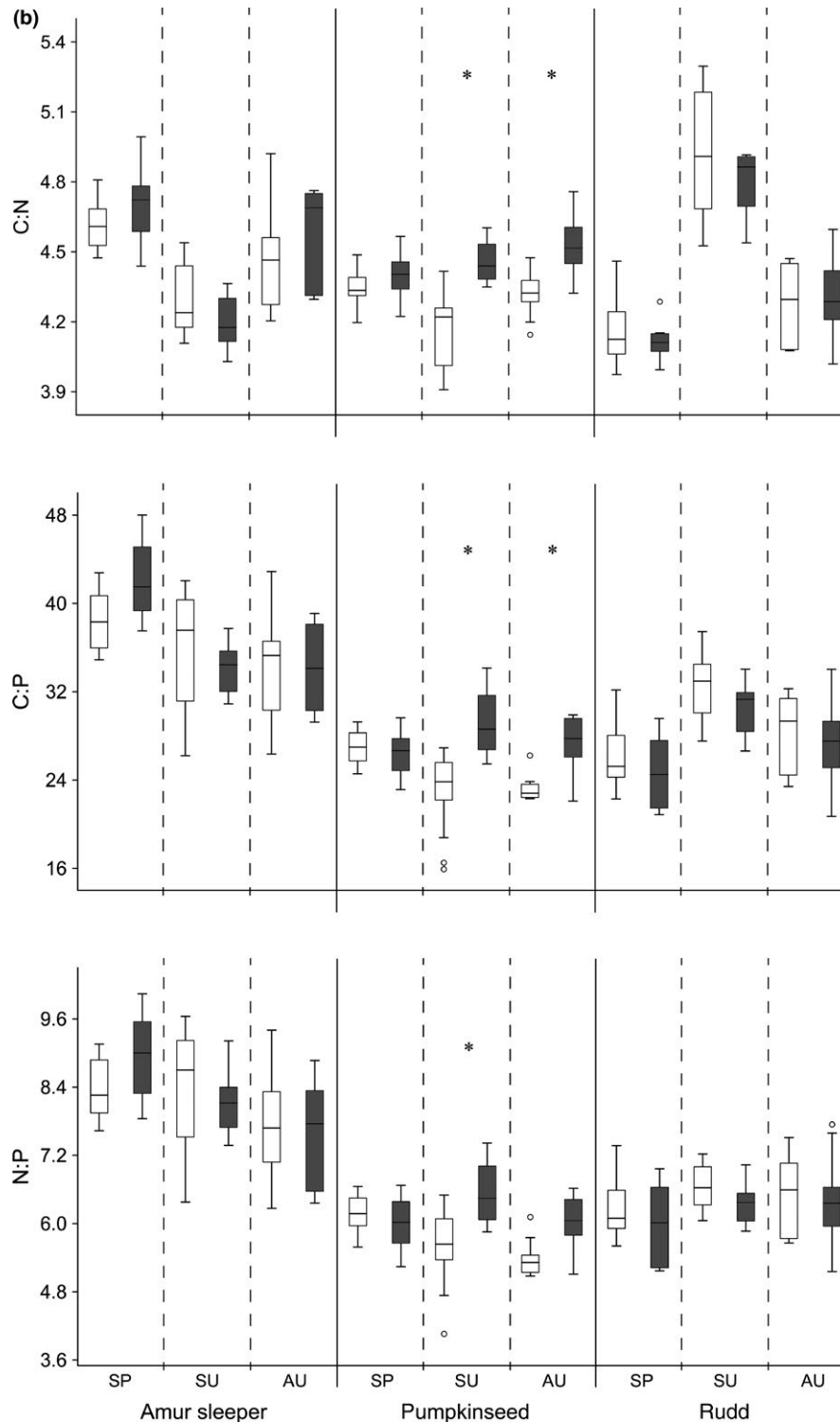


FIGURE 2 (Continued)

(Pizzolon et al., 2012). This results in a decline in %C independent of gonadal development. On the other hand, the moderate variability of %L suggested that the non-L resources (i.e. carbohydrates and protein) may play a substantial role in energy storing in these species. Both nest-guarding and the utilisation of non-L energy storages may have caused the differences in OS between sexes independent of GSI and %L.

Seasonal variation in both %P and N:P was much greater than for %C, suggesting that the changes in these two parameters could not be simply due to the diluting effect of seasonal %L fluctuations. The effect of reproductive allocation in this complex and broad seasonal OS variability cannot be ruled out. However, the species- and OS trait-specific influence of GSI indicated that the effect of seasonality on OS extends beyond gonadal development-derived changes. Previous studies on

the OS of fish reported that such complex effects on OS traits can be induced by somatic growth (Boros et al., 2015; Pilati & Vanni, 2007; Tanner, Brazner, & Brady, 2000). In fast-growing species, the proportionally faster development rate of muscles (i.e. the main N pool) compared to other tissues or macromolecules that serve as pool for other nutrients (e.g. bones for P and L for C) can generate interspecific OS variability (Tanner et al., 2000). More generally, the different increment dynamics of the main pooling tissues (adipose, muscle and bone tissues) during intense growth can lead to remarkable changes in OS. Similar patterns were observed during early ontogeny of fish, resulting in intraspecific OS variability as great as the interspecific variance (Pilati & Vanni, 2007; Boros et al., 2015). By considering this linkage between somatic growth and OS, and the substantial changes in somatic growth rate throughout the year (Shuter et al., 2012), we suspect that somatic growth may affect seasonal OS variability in fish. The species-specific developmental dynamics of main nutrient pooling tissues (Boros et al., 2015) may contribute to interspecific differences in patterns of seasonal OS variability.

Similarity in seasonal variation of TL and OS traits was not observed, indicating that the changes in the size of energy reserves and somatic growth may be more important in shaping OS than allometric changes. However, it has to be noted that although our study covered a wide size range, all sampled individuals were adults. Allometries of OS traits are substantial during early ontogeny (Boros et al., 2015; Pilati & Vanni, 2007), but it is getting less pronounced once fish reach their adult stage, which may explain the lack of linkage between TL and body composition in our study. Nevertheless, the effect of inter-individual variability in intensity and/or stage of somatic growth (i.e. differences in increment of main pooling tissues among individuals; Tanner et al., 2000) on OS cannot be ruled out.

Although interspecific differences in OS are well studied, there is still much to learn about the extent of intraspecific differences in OS (El-Sabaawi et al., 2014), which could clarify the importance of intraspecific OS variabilities in consumer-driven nutrient dynamics (Villéger, Brosse, Mouchet, Mouillot, & Vanni, 2017). By simultaneously examining the intra- and interspecific OS variability, we confirmed that the extent of intraspecific OS changes can exceed the interspecific differences: the seasonal variabilities of %C, %N and C:N within a species were greater than interspecific differences in the same parameters. However, the extent of intraspecific variability in %P, C:P and N:P was minor compared to interspecific ones. Interspecific differences in C and N content are frequently more narrow than those of P (e.g. El-Sabaawi et al., 2014; Hendrixson et al., 2007; Sterner & Elser, 2002). We observed interspecific variability in %C and %N one order of magnitude smaller than those in %P. This substantially broader difference in P content potentially contributes to the high species dependence of P content and P-related OS traits. The difference between fish species in their body P content arises from the difference in their anatomy and skeletal/bony structures; these traits are rooted deeply in the phylogeny of species (Hendrixson et al., 2007). Our findings provide additional evidence that the spiny-rayed fish species with ctenoid scales (such as the centrarchid pumpkinseed) exhibit higher P content than the soft-bodied species (like the cyprinid rudd; Hendrixson et al., 2007; Sterner & George, 2000). Although the most pronounced intraspecific

differences frequently occur in P content and P-related OS traits (El-Sabaawi, Kohler et al., 2012; El-Sabaawi, Travis et al., 2012; Pilati & Vanni, 2007), these traits are determined primarily by species identity.

In conclusion, we have demonstrated substantial seasonal variation in fish OS and showed that intraspecific variability can exceed the interspecific differences. In the light of these findings, studies on intraspecific variability should be expanded to include the temporal scale and the simple species-specific approach probably should be reconsidered in studies on elemental composition of animals and in ecological stoichiometry. Furthermore, such large intraspecific OS variability highlights its potential relevance in consumer-driven nutrient dynamics. Further studies should clarify how such seasonal fluctuations in elemental content (i.e. nutritional demand) modify the excretion patterns and the role of fish in nutrient dynamics.

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