



Different impacts of diet composition on the stoichiometric traits of two freshwater species

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Abstract Body elemental composition of consumers is a crucial parameter linking organisms' attributes to environmental changes. Recent investigations have revealed substantial intraspecific variability in organismal stoichiometry, challenging the assumption that individuals within a population have similar elemental composition. Yet, disentangling the factors

that promote intraspecific variation in organismal stoichiometry remains important. Here, we experimentally assessed the effect of diet elemental composition on the stoichiometric traits [percentage and ratios of carbon (C), nitrogen (N) and phosphorus (P)] of two omnivorous species, the red swamp crayfish (*Procambarus clarkii*) and the pumpkinseed sunfish (*Lepomis gibbosus*). Sunfish exhibited 6 times higher P and 1.6 times higher N contents than crayfish. Diet composition was an important driver of organismal stoichiometry variation within species, but its effect was also taxon-dependent. Our study revealed that the effects of diet variability on intraspecific stoichiometric traits are important but also contingent on the taxonomy.

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Introduction

Ecological stoichiometry investigates the balance between multiple elements in ecological interactions. It provides a unique framework to assess the elemental requirements of organisms and element available in their diet, aiming to understand the role of organisms on ecosystem functioning (Sturner and Elser 2002; Welti et al. 2017). Organismal stoichiometry refers to the ratios of elements stored in body tissues,

providing an assessment of the role of organisms in element storage and ultimately remineralisation via carcass decomposition (Sturner and Elser 2002; Boros et al. 2014). Therefore, revealing the drivers of organismal stoichiometry is fundamental to predict their implications in ecosystem-level biogeochemistry.

Variation in organismal stoichiometry can be driven by phylogeny because distinct evolutionary histories are expected to drive distinct structural characteristics, hence different elemental requirements (Hendrixson et al. 2007; González et al. 2018; Allgeier et al. 2020; Andrieux et al. 2021; Atkinson et al. 2020). Vertebrate and invertebrate species within the same feeding group can vary in their stoichiometric traits due to structural differences. Accounting for the taxonomic identity (vertebrate versus invertebrate) may help explaining counter-intuitive patterns of organismal stoichiometry observed within-species. Indeed, growing evidence has accumulated on the existence of substantial within-species variation in stoichiometry traits, with a variety of drivers involved (e.g. environment-induced: El-Sabaawi et al. 2012a; El-Sabaawi et al. 2012b; genetically-induced: Prater et al. 2017; ontogeny-induced Villar-Argaiz et al. 2020; combination: Vrede et al. 2011). Focusing on intraspecific variation is crucial because stoichiometric traits modulate the response of consumers to environmental changes (functional response trait), but also their impacts on ecosystems (functional effect trait; Díaz and Cabido 2001; Leal et al. 2016). Thus, the dual role of elemental composition makes it central in the dynamics of populations and ecosystems. Although intraspecific variation has become a fundamental topic in organismal stoichiometry (Jeyasingh et al. 2014; Lemmen et al. 2019), determining its magnitude and drivers is still needed.

The amount of carbon (C), nitrogen (N) and phosphorus (P) stored in organisms often relies on diverse factors. Heterotroph organisms have long been considered to be able to maintain consistent body elemental composition (Sturner and Elser 2002), but several studies showed that they can vary widely in their degree of elemental homeostasis (Persson et al. 2010; Benstead et al. 2014). In particular, organisms' elemental composition is expected to vary during ontogeny (Halvorson and Small 2016). Diet elemental composition also represents a parameter that could explain, at least partially, body elemental variation

among consumers (Boros et al. 2015). Although intraspecific diet variation is ubiquitous across animal taxa (Araújo et al. 2011), understanding how it modulates organismal stoichiometry remains complex and this is particularly true in wild populations, because it requires to quantify the quality of multiple trophic resources consumed independently and mixed (see also alternative methods in Cross et al. 2007; Durston and El-Sabaawi 2019). This is even more challenging when considering omnivorous species that consume resources at multiple trophic levels (van der Velde et al. 2006). Correlation between elements can help understand the degree of linkage between elements. For instance, negative correlations between elements can highlight elemental mass-balance whereby increases in one element result in the reduction of another element (Hendrixson et al. 2007; Allgeier et al. 2020). Overall, organismal stoichiometry is likely determined by simultaneous drivers occurring at the individual (e.g. intraspecific diet variation) and taxonomic (e.g. variation in elemental trait stoichiometry due to distinct body plans) levels. Therefore, it is important to quantify the extent to which the effects of intraspecific diet variability are contingent on the taxonomy.

In this study, we experimentally investigated the role of taxonomy (vertebrate versus invertebrate) and diet elemental composition on organismal stoichiometry in the red swamp crayfish (*Procambarus clarkii*) and the pumpkinseed sunfish (*Lepomis gibbosus*). Red swamp crayfish and pumpkinseed sunfish were selected as model species because they are opportunistic omnivores with a wide diet variability (Evangelista et al. 2017; Jackson et al. 2017) and have structural differences (endoskeleton versus exoskeleton) that are likely associated with distinct scaling patterns relating body mass and skeletal tissues. These differences in body plans between vertebrates and invertebrates ultimately imply distinct nutritional requirements (Hendrixson et al. 2007). In the laboratory, specimens of each species were individually fed with diets differing in the quality and diversity of food items (Evangelista et al. 2017; Fig. 1). To avoid any ontogeny effect, we used individuals with similar body size for each taxa and we tested the effects of diet elemental composition and taxonomy on organismal stoichiometry (percent composition and ratios) in controlled conditions.

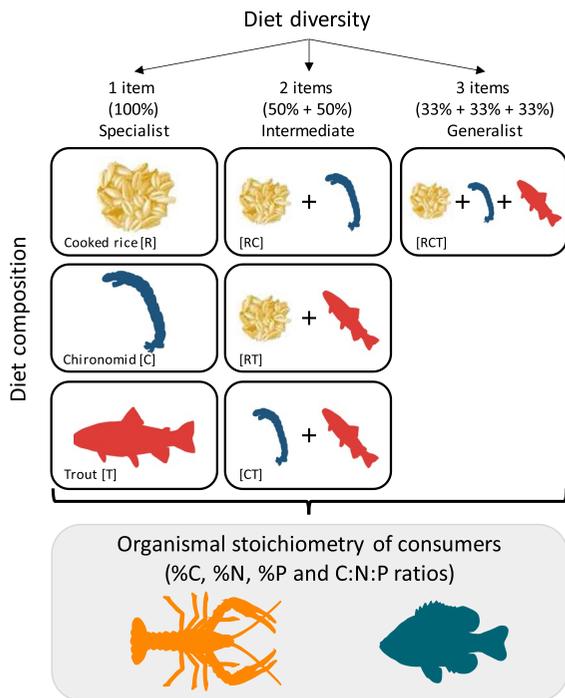


Fig. 1 Schematic representation of the experimental design used to test the effect of diet elemental composition and taxonomy on organismal stoichiometry in two omnivorous species (*Procambarus clarkii* and *Lepomis gibbosus*). Diet was manipulated to mimic the full range of diets observed in wild populations. Specifically, diet treatments varied both in term of diversity (one, two or three food items leading to specialist, intermediate and generalist consumers, respectively) and resource composition. Diet treatments were created using three items representing plants (cooked white rice [R]), macro-invertebrate (chironomid [C]) and fish (grounded rainbow trout dorsal muscle with skin [T]). A total of seven diet treatments was used

Specifically, we tested the following predictions: (1) individuals feeding on a nutrient-rich diet will have higher N and P contents, and lower C:N and C:P ratios than individuals feeding on nutrient-poor diet (McIntyre and Flecker 2010), regardless of their taxonomic identity; (2) the effects of resource composition on stoichiometric trait will be taxon-dependent (González et al. 2018; Atkinson et al. 2020), and red-swamp crayfish will have lower N and P contents and higher N:P ratios contents than pumpkinseed sunfish due to the structural variations between fish and crustacean (P-rich bones versus N- and P-rich exoskeleton, Elser et al. 1996; Sterner and Elser 2002).

Material and methods

Sampling and rearing of experimental individuals

Pumpkinseed sunfish (hereafter referred to as sunfish) and red-swamp crayfish (hereafter referred to as crayfish) are both opportunistic omnivores and forage on animals (e.g. invertebrates and fish) and plant material (e.g. algae, macrophytes, wind-spread seeds and detritus), although sunfish have a more animal-based diet than crayfish (Jackson et al. 2017; Gkenas et al. 2019). These two species were selected as model species because they can have a pivotal role in aquatic ecosystems (Oficialdegui et al. 2019; Yavno et al. 2020).

Specimens used in the present study originated from a single gravel pit lake (43°23'9.6"N; 1°15'57.6"E) located along the flood plain of the Garonne River in southwestern France where the two species are abundant and often coexist (Evangelista et al. 2015, 2019; Alp et al. 2016; Jackson et al. 2017). Sunfish and crayfish were collected in the littoral habitat of the lake using a seine net (5-mm mesh size) or fishmeal pellets baited traps (rectangular trap: 95 cm × 20 cm × 20 cm, 4-mm mesh size), respectively (Evangelista et al. 2017, 2019). Sunfish were collected on October 3rd, 2013 and crayfish on September 17th, 2015. They were then transported to the laboratory where they were acclimated to laboratory conditions (photoperiod: 12/12 h; water temperature: 17 to 18.5 °C, as experienced by specimens in the studied lakes at the same period of the year; Garcia et al. 2023) over six (sunfish) and five weeks (crayfish) before the start of the experiment. During the acclimation period, sunfish were kept in three 200-L tanks, while crayfish were maintained individually in 50-L tanks. All individuals were fed *ad-libitum* with commercial red maggots (*Diptera*) until the start of the experiment to minimize background variability in body elemental composition among individuals.

Laboratory experiments

The two experiments involving sunfish and crayfish were conducted separately, i.e. from 15 November 2013 to 16 February 2014 (9 weeks) and from 21 October to 16 December 2015 (8 weeks), respectively. The two experiments followed the same experimental procedure (Evangelista et al. 2017). At

the end of the acclimation period, individuals were measured for initial fork or carapace length (FL_i or $CL_i \pm 1$ mm) and weighed ($W_i \pm 0.1$ g). Then, for each species, individuals of similar size were selected for the experiments (48 sunfish: FL_i range 79–98 mm, mean \pm SD: 89.0 ± 5.3 ; 40 crayfish: CL_i range 36.7–45.3 mm, mean \pm SD: 41.5 ± 2.0). Experimental individuals were of similar size and, for each species, size range was relatively narrower than the size range observed in natural populations in the studied area (e.g. FL range 65–148 mm, mean \pm SD: 102.3 ± 18.0 ; CL range 19.6–64.7 mm, mean \pm SD: 45.6 ± 7.4 ; personal data). Hence, the effect of ontogeny on organismal stoichiometry was limited in our experiment (Pilati and Vanni 2007). After measurement, sunfish were transferred individually to 50-L tanks while crayfish were released into their respective tank. Each tank was equipped with a mechanical filtration system, a plastic plant and a shelter. The experimental units (i.e. 48 sunfish and 40 crayfish) were randomly distributed among six or five shelving units containing eight treatments each. Individuals were randomly assigned to one treatment (see details below) and there was no significant difference in the mean weight and length at the start of the experiment between treatments (ANOVAs; FL_i Sunfish: $F_{6, 41} = 2.18$, $p = 0.065$; WL_i Sunfish: $F_{6, 41} = 1.58$, $p = 0.178$; CL_i Crayfish: $F_{6, 32} = 0.11$, $p = 0.995$; W_i Crayfish: $F_{6, 32} = 0.14$, $p = 0.989$).

Each individual was hand-fed with one of the seven diet treatments that allowed to mimic the full extent of diets observed in wild populations (Jackson et al. 2017; Evangelista et al. 2017) and three levels of decreasing trophic specialization (Bolnick et al. 2003). These diets varied in both diversity (one, two or three food items) and elemental composition (Fig. 1, Supplementary S1) and were created using three items representing vegetable matter [cooked white rice (R)], macro-invertebrate [chironomid (C)] and fish [grounded rainbow trout dorsal muscle with skin (F)]. The seven diet treatments included three types of specialists (*sensu* Bolnick et al. 2003) feeding on a single diet item (R, C or F), three types of intermediate feeding on a mixture of two diet items (RC, RF or CF) and one generalist type feeding on all the diet items (RCF) (Bolnick et al. 2002; further details available in Evangelista et al. 2017).

The three items were selected as they represent the three different reported trophic levels of

prey consumed by the model species and cover a broad range of elemental content (%C, %N and %P; Fig. S1.A), enabling to mimic natural variation in food stoichiometry experienced by wild sunfish and crayfish. White rice was selected as a plant-based source because it is readily available in a standardized size and quality. For each model species, chironomid and fish items were bought from the same companies to limit variation in the content of food between the two experiments. A daily ration of 3% of individual body mass was used to prepare the diet (wet mass) of each individual (Glaholt and Vanni 2005). For mixed diets (i.e. RC, RF, CF and RCF), the total mass of each item was partitioned equally among items, and diets were homogenized manually to limit the bias toward the consumption of preferred item(s). All treatments were replicated six times for sunfish or five times for crayfish, respectively, except RCF which was replicated twelve or ten times (for sunfish and crayfish, respectively) to account for higher variability in the mixture that arose from the combination of three items. At the end of the experiments, individuals were euthanized with an overdose of anesthetic (benzocaine). One crayfish fed with trout meat died during the experiment and was not used in the subsequent analyses.

Diet and organismal stoichiometry

Organismal stoichiometry was quantified from gutted sunfish and whole crayfish fasted for 48 h, and duplicate subsamples were analysed for each individual. For all stoichiometric analyses, resource and consumer (whole individual) samples were freeze-dried and ground to a homogeneous powder. Subsamples were analysed for % C and % N of dry mass using a CHN analyser (Flash 200, Thermo Fisher Scientific, Waltham, MA, USA; method ISO 10694:13878), and for % P following the molybdate-blue method using a spectrophotometer (Uvi Light XT5, Secomam, Alès, France; absorbance measured at 880 nm) after a sulfuric acid (15N) digestion in autoclave. Samples of a standard reference material (yeast, Internal Standard) were used to validate the efficiency of P recovery of this method (96.5%). Consumer and resource element contents were used to calculate molar ratios for C:N, C:P and N:P. For the crayfish experiment, resource element contents of each treatment were quantified using five (single diet) or three (mixture) replicates.

For the sunfish experiment, resource element contents were quantified for each single treatment (R, C and T) using five replicates. Then, element contents of the different mixed diets (RC, RT, CT and RCT) were estimated using the element content of each single diet partitioned equally. On average, dry mass percentage of diet composition varied from 38.32 to 56.82 in %C, from 1.49 to 11.05 in %N and from 0.23 to 0.89 in %P (Fig. S1.A).

Statistical analyses

All statistical analyses were performed using R v.4.0.3 (R Development Core Team 2020). To avoid collinearity among predictive variables, the variability in percent composition of the resource (%C, %N and %P) was synthesized into a multidimensional space using a principal component analysis (PCA). PCA was conducted using the *FactoMineR* package (v. 2.4; Le et al. 2008) and individual diet values were isometric log-ratio transformed, as recommended for compositional data (Jackson et al. 1997; Egozcue et al. 2003). The transformation was performed using the *ilr* function from the *compositions* package (v.2.0.6; van den Boogaart et al. 2024). The first PCA axis (diet PC1; variance explained > 10% and eigenvalues > 1) was used as a composite variable in subsequent analyses (Table S2.A).

For each species, covariations between body elemental composition among individuals were tested using Spearman correlations. Linear models (one for each stoichiometric trait) were used to test the effects of taxonomy (species), diet composition (diet PC1) and their interaction on the elemental composition (%C, %N, %P) and elemental ratios (C:N, C:P, N:P) of crayfish ($n=39$) and sunfish ($n=48$). The significance of each explicative variable was estimated using the *Anova* function from the *car* package (v.3.0.10; Fox and Weisberg 2011). Specifically, Type III sum of squares were calculated when the interaction was significant, while Type II sum of squares were extracted when the interaction was not significant (Langsrud 2003). The interaction was maintained in the final models, whether it was significant or not. Post-hoc analyses on the significant interactions were performed using the *emmeans* function from the *emmeans* package (v.1.5.2.1; Lenth 2020). *P* values were adjusted using the false discovery rate procedure using the *p.adjust* function. All stoichiometric

ratios (C:N, C:P and N:P) were log-natural transformed prior to analyses to ensure robust and reproducible results (Isles 2020). Assumption of linearity and homogeneity of variances on residuals of all linear models were checked using residuals analyses and diagnostic plots. %P was log-natural transformed to prevent serious deviation from normality.

Results

Diet stoichiometry and general patterns of body elemental composition

The first PCA axis explained 97.7% of the total variance across all diets (Fig. 2). Low PC1 values (diet PC1) were associated to high %C while high PC1 values were associated with high %N and %P (Fig. 2, Table S2.A).

Body P content was the most variable element in both species, (coefficient of variability $CV_{\text{crayfish}}=15.7\%$ and $CV_{\text{sunfish}}=19.1\%$; Table 1). Body N content was more variable in crayfish ($CV_{\%N}=13.0\%$) than in sunfish ($CV_{\%N}=4.1\%$; Table 1), followed by a lower variability in body C content ($CV_{\%C}=8.0\%$ and 6.3% , respectively). For both species, %N and %C were positively correlated (for crayfish: $P<0.001$, $\rho=0.62$; for sunfish: $P=0.025$, $\rho=0.32$; Fig. 3A-B). For crayfish, %C and %P, and %N and %P were positively correlated ($P=0.028$, $\rho=0.35$ and $P<0.001$, $\rho=0.54$, respectively; Fig. 3c-e). For sunfish, there was a negative correlation between %C and %P ($P<0.001$, $\rho=-0.51$; Fig. 3d), but %N and %P were not significantly correlated ($P=0.074$, $\rho=-0.26$; Fig. 3f).

Drivers of stoichiometric traits

Species was the most influential predictor of all stoichiometric traits (average $\eta^2=0.88\pm 0.15$ SD; Table 2). Sunfish were significantly C-, N- and P-enriched compared to crayfish (Table 1; Fig. 4). On average, sunfish were more than 6 times P-enriched than crayfish, while differences in %C and %N were more limited between the two species (1.2 and 1.6 times, respectively; Table 2; Fig. 4). Sunfish displayed significantly lower C:P, C:N and N:P ratios than crayfish (Table 2; Fig. 4).

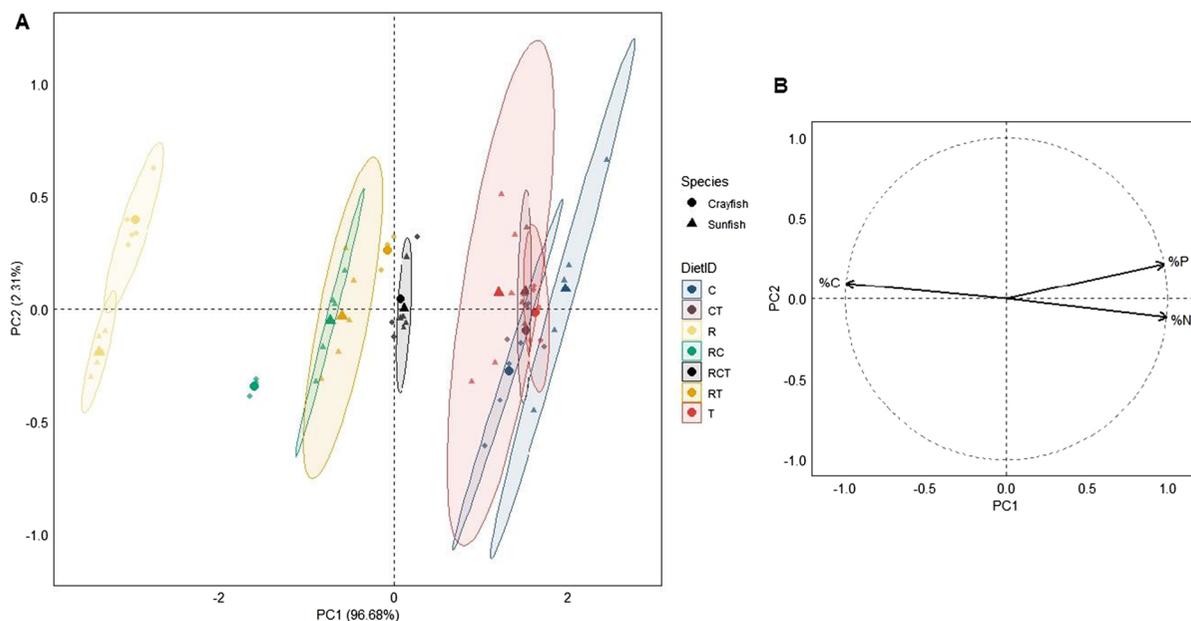


Fig. 2 Results of the principal component analysis (PCA) of the elemental composition (%C, %N, %P; isometric log-ratio transformed) of the seven diet treatments used in the experiments with crayfish (circle) and sunfish (triangle). Diet abbreviations correspond to rice [R], chironomid [C] and fish [T]. Ellipses represent 95% CI and large symbols are mean values used in the subsequent analyses

Ellipses represent 95% CI and large symbols are mean values used in the subsequent analyses

Table 1 Summary statistics of elemental composition of crayfish and sunfish individuals used in the study. Molar elemental ratios are displayed

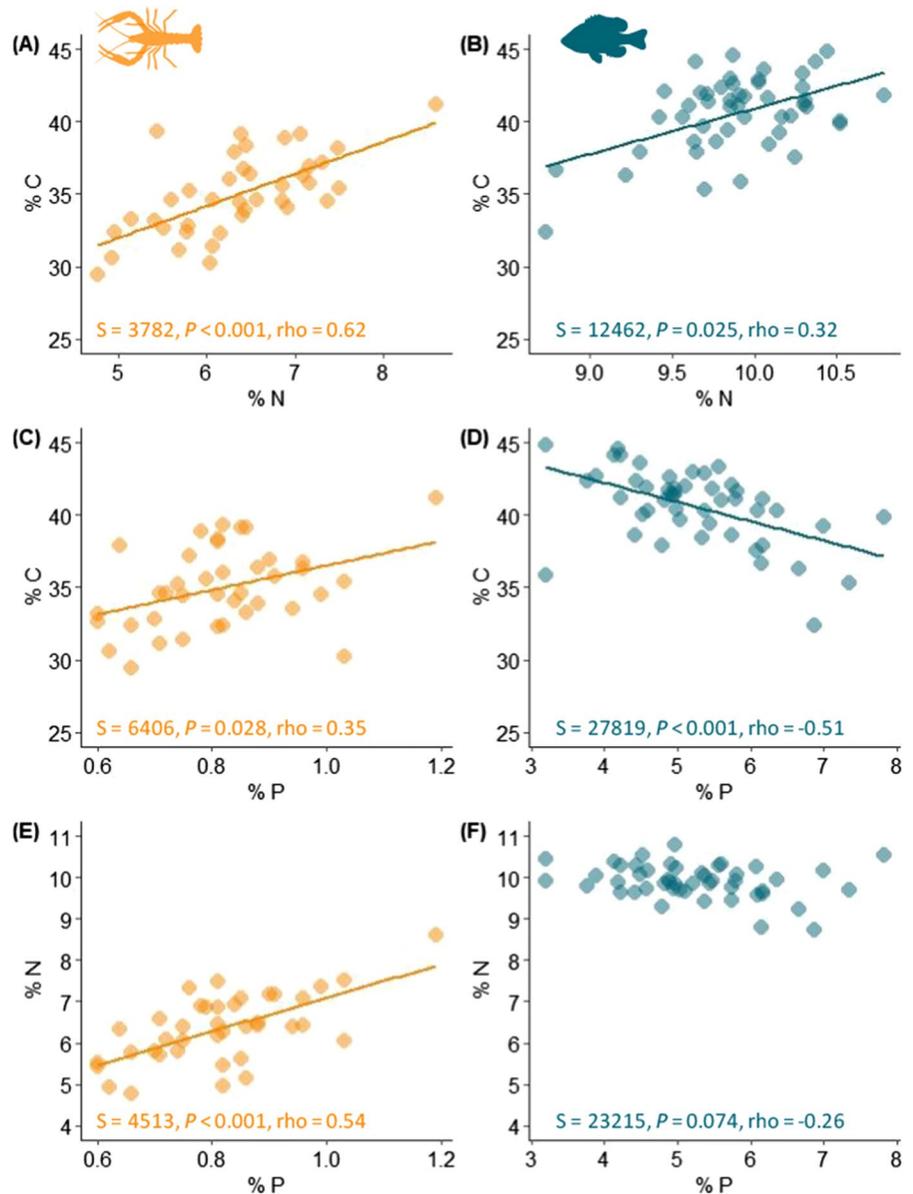
For each element and ratio, mean, standard error (SE), minimum (Min), maximum (Max) values and coefficient of variability (CV) are provided

	%C		%N		%P	
	Crayfish	Sunfish	Crayfish	Sunfish	Crayfish	Sunfish
Mean (SE)	35.0 (0.45)	40.6 (0.37)	6.3 (0.13)	9.9 (0.06)	0.8 (0.02)	5.2 (0.14)
Min	29.4	32.4	4.8	8.7	0.6	3.2
Max	41.2	44.8	8.6	10.8	1.2	7.8
CV (%)	8.0	6.3	13.0	4.1	15.7	19.1
	C:N		C:P		N:P	
Mean (SE)	6.5 (0.11)	4.8 (0.04)	112.7 (2.59)	20.9 (0.70)	17.4 (0.36)	4.3 (0.13)
Min	5.5	4.2	75.9	12.2	13.0	2.8
Max	8.4	5.3	151.8	36.2	21.7	7.2
CV (%)	10.1	5.6	14.2	23.1	12.6	21.6

Except for N:P, the effects of diet composition on the stoichiometric traits of consumers were taxon-dependent as revealed by the significant interactions between Species \times Diet PC1 (Table 2; Supplementary S3). For crayfish, both %N and %P contents significantly increased with increasing diet PC1 values (Fig. 4b, c), indicating that crayfish that consuming N- and P-enriched diet had a higher %N and %P content than crayfish consuming diet containing low %N and %P. Percent C of crayfish did not differ

significantly between diet composition. Accordingly, C:N ratios of crayfish significantly decreased with increasing diet PC1 values (Fig. 4d). Similarly, %N of sunfish significant increased with increasing diet PC1 values (Fig. 4b), and the same pattern was observed for %C (Fig. 4a). C:P ratios of sunfish were also significantly and positively influenced by diet PC1 values (Fig. 4e). This indicates that individuals consuming N- and P-enriched food had a higher C:P ratio (i.e. high C relative to P). N:P ratios of both species

Fig. 3 Correlations between elemental composition (%C, %N and %P). Each colour represents a species: crayfish (orange; $n=39$) and sunfish (blue; $n=48$). The coefficient of correlation (ρ), P value and test value are displayed in each panel. Significant correlations are displayed using regression lines



significantly increased with increasing diet PC1 values (Table 2; Fig. 4f).

Discussion

Variation in organismal stoichiometry is common in nature and can occur both at the interspecific and intraspecific levels. Yet, understanding organisms' stoichiometric variability in the wild is extremely complicated, especially due to the implications of

numerous confounding factors (Halvorson and Small 2016). Here, we investigated the drivers of inter- and intra-specific variabilities simultaneously in controlled conditions. Specifically, we tested the effects of taxonomy, diet elemental composition and their interactions in modulating the stoichiometric traits of two omnivorous species that are taxonomically distinct. We found evidence that individuals feeding on N-rich resources displayed high body N contents. In turn, these individuals displayed low C:N ratios (i.e. high N relative to C), at least for crayfish because C:N

Table 2 Analysis-of-deviance table derived from the linear models used to test the effects of species and diet composition (PC1 values) on elemental compositions (%C, %N and %P) and molar elemental ratios (C:N, C:P and N:P) of crayfish ($n=39$) and sunfish ($n=48$)

Predictors	% C			% N			% P (log-natural)					
	SS	F_{df}	adjP	η^2	SS	F_{df}	adjP	η^2	SS	F_{df}	adjP	η^2
Intercept	47,628	7998.35 ₁	<0.001	-	1576.16	6231.90 ₁	<0.001	-	1.78	59.93 ₁	<0.001	-
Species	673	113.04 ₁	<0.001	0.58	268.37	1061.10 ₁	<0.001	0.93	73.76	2485.48 ₁	<0.001	0.97
Diet (PC1)	2	0.30 ₁	0.588	0.14	10.05	39.72 ₁	<0.001	0.34	0.12	4.11 ₁	0.046	<0.01
Species × diet (PC1)	35	5.81 ₁	0.024	0.07	1.83	7.23 ₁	0.009	0.08	0.21	7.09 ₁	0.012	0.08
Residuals	494	- ₈₃	-	-	20.99	- ₈₃	-	-	2.46	- ₈₃	-	-
Predictors	C:N (log-natural)						N:P (log natural)					
	SS	F_{df}	adjP	η^2	SS	F_{df}	adjP	η^2	SS	F_{df}	adjP	η^2
Intercept	135.33	39,155.84 ₁	<0.001	-	865.45	24,860.90 ₁	<0.001	-	-	-	-	-
Species	1.93	559.31 ₁	<0.001	0.87	62.26	1788.60 ₁	<0.001	0.96	42.29	1437.80 ₁	<0.001	0.95
Diet PC1	0.23	65.42 ₁	<0.001	0.15	0.09	2.62 ₁	0.109	0.02	0.22	7.39 ₁	0.012	0.08
Species × diet (PC1)	0.19	53.68 ₁	<0.001	0.39	0.36	10.39 ₁	0.002	0.11	0.03	0.99 ₁	0.322	0.01
Residuals	0.29	- ₈₃	-	-	2.89	- ₈₃	-	-	2.44	- ₈₃	-	-

Stoichiometric ratios and %P were log-natural transformed. Significant P values adjusted for the false discovery rate (adjP) are displayed in bold. Variance decomposition (eta-squared η^2) was estimated for each variable

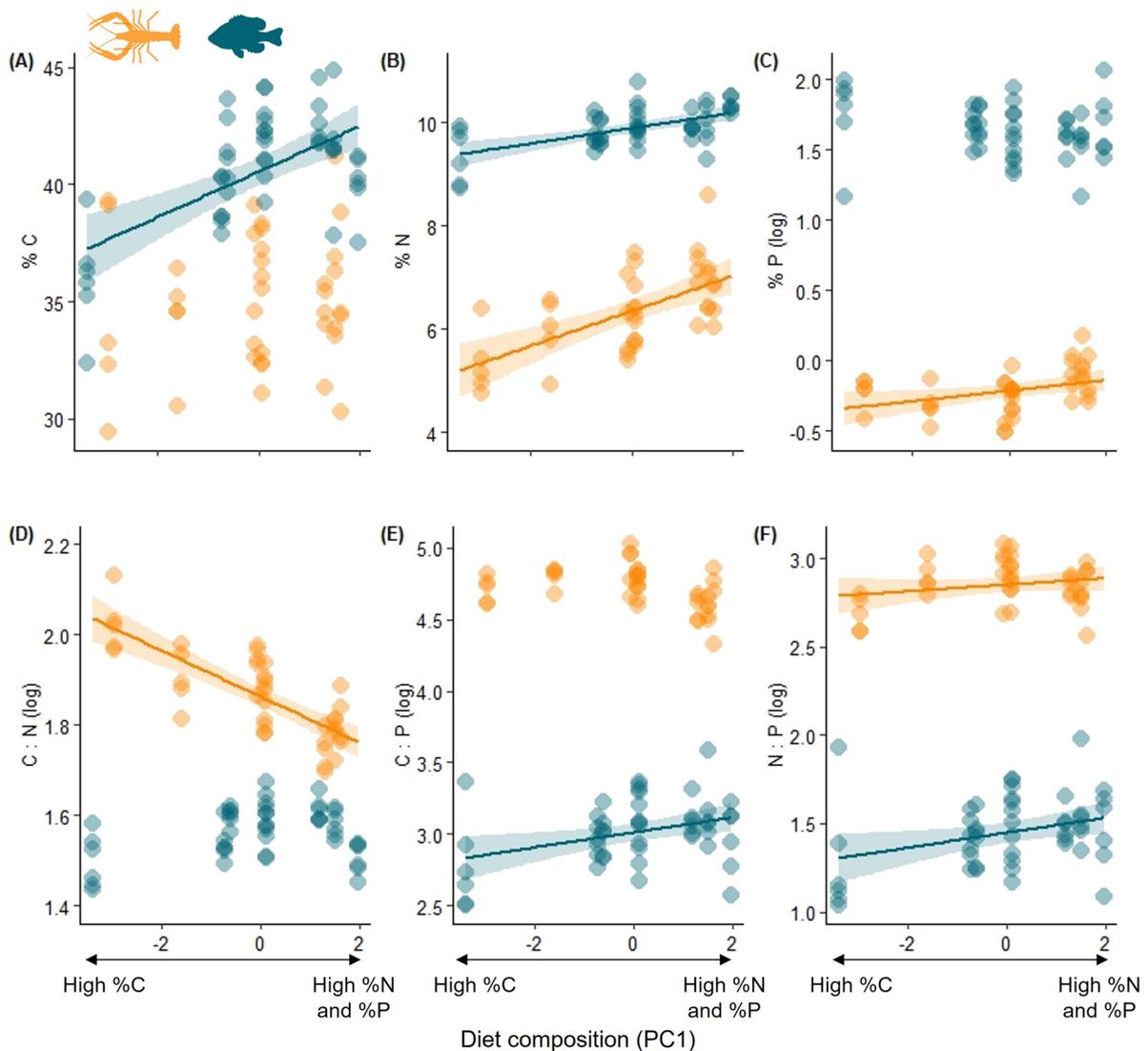


Fig. 4 Relationships between diet composition (PC1) and stoichiometric traits (%C, %N, %P, C:N, C:P and N:P; log natural transformed, except %C and %N) in crayfish (orange; $n=39$)

and sunfish (purple; $n=48$). Significant relationships are displayed using regression lines. Grey shaded areas are 95% CI

ratios of sunfish remained constant. Body P content of crayfish also responded to contrasting diet stoichiometry, while %P of sunfish did not vary with diet stoichiometry. The opposite pattern was observed for body C and C:P, where only those of sunfish significantly increased with nutrient-enriched diet. These findings demonstrate that the effects of resource composition on organismal stoichiometry were contingent on the taxonomic identity of the specimens (second prediction). Finally, we found that crayfish were

both N- and P-depleted and had higher N:P ratios compared to sunfish, which is consistent with our prediction.

Both crayfish and sunfish species displayed intraspecific variation in elemental body composition. It is important to highlight that individuals used in the present study had a narrow body size range; therefore, intraspecific variability was likely underestimated because body size can explain a large proportion of variation in stoichiometric traits (González

et al. 2018). Although it is hard to compare our coefficient of variability to those published in the literature because different methods are used for element determination (Boros and Mozsár 2015), our results show that body N content of crayfish was 3 times more variable than body N content of sunfish, while variability in C and P were similar between species. In the present study, we found that body P content was the most variable element for both crayfish and sunfish, though diet could not influence patterns of body P content of sunfish. This likely indicates that other mechanisms may partially explain elemental variability in this species. One mechanism, for instance, would be the differences in nutrient assimilation efficiency, but this will require further investigations. Altogether, these findings indicate that although intraspecific variability in elemental composition occurs ubiquitously across animals (González et al. 2011; Moody et al. 2018; Lemmen et al. 2019; Rizzuto et al. 2019), more studies are needed to understand the magnitude of intraspecific variability in other traits such as assimilation efficiency (Lemmen et al. 2019).

Diet composition appeared to be a key driver of organismal stoichiometry, but this effect was often taxon-dependent. This indicates that understanding how ecological factors modulate organismal stoichiometry requires a good knowledge of species-specific elemental requirements. Crayfish and sunfish are omnivorous but crayfish have a more plant-based diet than sunfish (Jackson et al. 2017; Gkenas et al. 2019), and thus consume more resources with high C:nutrient ratios. Our results demonstrated that crayfish have higher C:nutrients ratios than sunfish. Crayfish also had lower %N than sunfish, as already reported when comparing detritivores and predators (Lemoine et al. 2014; González et al. 2018). Our results may indicate that specimens changed their elemental composition to minimize elemental mismatch with their diets. However, disentangling the mechanisms underpinning species-dependent variations in organismal stoichiometry (e.g. genetic- and/or trophic-driven effects; Prater et al. 2017) will require more investigations. In fact, differences in elemental composition between species are likely explained by structural differences between arthropods and vertebrates. For instance, crayfish were N-depleted compared to sunfish, perhaps because N-demand for chitin production remains lower than N-demand for muscle production.

Almost all elements covaried, but the direction of these correlations differed between the two species tested. In general, we observed positive correlations, as already reported in previous studies (Cai et al. 2016; Allgeier et al. 2020; Andrieux et al. 2021). Furthermore, our results were consistent with the literature when considering invertebrate specimens (i.e. positive correlations for all pairs; Allgeier et al. 2020; Andrieux et al. 2021) and likely confirmed our expectation that exoskeleton of arthropods, especially crustaceans, requires higher amounts of all elements. For vertebrates, in contrast, Allgeier et al. (2020) observed negative correlations between C and P, and N and P, and a positive correlation between C and N. In the present study, we found a negative correlation of C and P in sunfish, no correlation for sunfish N and P, and a positive correlation for sunfish C and N, which partially match results reported for fish (Andrieux et al. 2021). This highlights that stoichiometric covariations can differ between and within taxonomic groups, and this is likely because different species within the same taxa often differ in their physiological constraints.

In conclusion, this study contributes to a growing body of research calling for considering intraspecific diversity in community and ecosystem ecology (Des Roches et al. 2018; Raffard et al. 2019). Focusing on variations in body elemental composition is important, because these traits (i.e. percent and ratios) inform on the responses of organisms to environmental changes (functional response trait), but also their role in ecosystems (functional effect trait). To identify the mechanisms driving intraspecific variability, future studies should also analyse variations in other stoichiometric traits (e.g. assimilation efficiency) that are predicted to display greater variability within species (Lemmen et al. 2019).

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Authors' contribution CE and JC conceived the ideas and design the methodology; CE and RL conducted the experiments; CE analysed the data; CE wrote the initial draft of the manuscript and JC and MD contributed critically to revisions. All authors approved the final version of the manuscript.

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Data and code availability Data and R codes used in this study are hosted in the Figshare repository (<https://doi.org/10.6084/m9.figshare.23822535.v1>, Evangelista et al. 2023).

Declarations

Conflict of interest There are no conflicts of interest to declare.

Ethical approval Authorizations to collect invasive specimens were provided by the “Arrêtés Préfectoraux—10/07/2013 and from 18/09/2015 to 30/10/2015”.

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