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Two is better than one: combining gut content and stable isotope analyses to infer trophic interactions between native and invasive species

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Abstract

Two complementary approaches are commonly used for analysing trophic interactions that allow inferences about consumed and assimilated resources: gut content (GCA) and stable isotope (SIA) analyses. We used these methods to assess the trophic ecology of a native (*Pontastacus leptodactylus*) and an invasive species (*Faxonius limosus*) of crayfish in the Lower Danube (Romania) by calculating five frequently used metrics (i.e. trophic position, omnivory index, elemental imbalance for C:N ratio, trophic niche width and overlap). The SIA approach indicated significantly different trophic niche widths between the crayfish species and potential resource partitioning that triggered lower niche overlap, aspects not depicted by GCA. The latter approach suggested higher omnivory indices and elemental imbalance for macronutrients, potentially because of high incidences of basal resources with low nitrogen content in the foreguts of both species. Although, as indicated by GCA, the two species seem to feed largely on the same resources, SIA suggested that the ingested food items were assimilated with differing efficiencies. These findings are of importance for studies of invasion biology, where the replacement of native species by invasive taxa is underpinned by mechanisms that should be explored by using the most appropriate approach.

Keywords

Principle of competitive exclusion
Trophic position
Omnivory index
Trophic niche width

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Introduction

The Principle of Competitive Exclusion predicts that interspecific interactions between species with overlapping trophic niches that occur within the same habitats will result in either extinction (Webb et al., 2002) or long-term coexistence (Pianka, 1973; Weber, 1999). The latter situation is usually associated with trophic niche shifts (Jackson & Britton, 2014), decreases in trophic position (Vander-Zanden et al., 1999) or with changes in elemental imbalance (i.e. dissimilarity in nutrient content between consumers and their food) for major macronutrients (González et al., 2010). In the context of biological invasions, the duration of coexistence is considered a key factor driving relevant ecological and evolutionary processes (Bøhn et al., 2008). It was showed that short-term (a few years) coexistence between invasive and native species with a similar trophic ecology induced strong niche overlap (Bøhn & Amundsen, 2001; Olsson et al., 2009). In contrast, long-term coexistence (more than a decade) was accompanied by shifts in diet and habitat segregations with important consequences for trophic interactions between the competitors (Brabrand & Faafeng, 1993; Sharma & Borgstrøm, 2008; Eloranta et al., 2013). For crayfish, for instance, competition between invasive and native species has often resulted in either the elimination of the latter (Olden et al., 2006; Olsson et al., 2009) or coexistence (Whitledge & Rabeni, 1997; Magoulick & Piercey, 2016). Where native and invasive crayfish species have managed to coexist, it is usually through niche segregation (Jackson et al., 2014), accompanied by multi-trophic effects on local biota (Fitzsimons et al., 2007; Johnson et al., 2009).

The assessment of trophic interactions between native and invasive crayfish is usually undertaken using gut content (GCA) or stable isotope (SIA) analyses (Olsson et al., 2008, 2009; Kadye & Booth, 2012). GCA represents one of the main approaches used, based on frequent sampling in aquatic habitats to quantify the diet of invasive and native crayfish (Marufu et al., 2017, 2018). One of the advantages of GCA is that it provides information about the taxonomy of the consumed food items, which is often not the case with SIA (Layman et al., 2012). However, it has the disadvantage of representing only a snapshot of the food consumed over a limited timeframe (Woodward & Hildrew, 2002). While, SIA provides an estimate of the biomass assimilated by consumers over the long-term (Bearhop et al., 2004), its usefulness can be limited, because in some cases even the latest state-of-art statistical tools (i.e. mixing models) struggle to provide good estimate of the dietary contribution of different prey to the diet of consumers (Moore & Semmens, 2008, Parnell et al., 2013). The reason for inaccurate estimations of these models may result from insufficient a priori knowledge of turnover rate of stable isotopes, trophic discrimination and macronutrient composition (e.g. free lipid and carbonate content) associated with the consumer tissues (Parnell et al., 2013). Due to, among other reasons, their differing temporal resolution, both techniques provide valuable information to characterise trophic interactions between competitive species (Rasmussen, 2010; Davis et al., 2012).

We used the complementary information provided by these two techniques to investigate two competing species in the Lower Danube. This river was successfully invaded by the spiny-cheek crayfish (SCC), *Faxonius limosus*

(Rafinesque, 1817), a North-American r-type reproductive strategist introduced in Europe for commercial purposes in 1890 (Filipová et al., 2011). After colonising the Lower Danube, SCC has expanded its range through the catchment, including the lowland sections of several tributaries (Puky & Schád, 2006). This invasion of the Danube, led to competition between SCC and the indigenous narrow-clawed crayfish (NCC), *Pontastacus leptodactylus* (Eschscholtz, 1823), a *K*-type reproductive strategist. The result was the near extermination of the native species from its natural habitats, such that NCC is now only present within the invaded section of the Danube as a remnant population in a small bay-like meander, where it has managed to coexist with the invasive species for at least a decade (Pârvulescu et al., 2015). The only other sector of the Danube where both species can still be found together is the invasion front (*sensu* Pârvulescu et al., 2015), comprising an area recently invaded (approximately three years) where the invasive SCC is rapidly replacing the native NCC.

Asymmetric competition leading to extirpation of one species (such as that between SCC and NCC) occurs when resources are exploited by the competitive species with greater efficiency, potentially driven by different metabolic rates, with implications for trophic niche width and overlap (Whitledge & Rabeni, 1997; Cleveland & Montgomery, 2003; Ho et al., 2007; Magoulick & Piercey, 2016). Moreover, due to the differences between ingested *versus* assimilated food, trophic metrics can differ between the GCA and SIA (cf. Rybczynski et al., 2008; Zambrano et al., 2010). Therefore, to assess the effects of time since coexistence on the trophic ecology of two competing species and the way the two different working techniques influence the understanding of interspecific interactions, we first determined their diet and several trophic metrics (i.e. the trophic position, omnivory index, elemental imbalance in C:N, trophic niche width and overlap) using both GCA and SIA, followed by comparisons across species and methodological approaches (including interactions in our models, as no information precluding species-specific differences between methods is available). We expected that long-term coexistence would involve shifts in diet, a low degree of niche overlap (Beatty, 2006; Jackson & Britton, 2014) and low niche widths for both competitors as a consequence of trophic segregation (Sjödin et al., 2018), accompanied by higher trophic position and omnivory for the invasive SCC (Jackson et al., 2014), and a lower elemental imbalance compared with the native NCC (González et al., 2010). At the invasion front, we expected a higher degree of niche overlap and similar values of trophic niche components between species due to their short-term coexistence ~~of species~~ (Bøhn & Amundsen, 2001; Olsson et al., 2009).

Materials and methods

Sampling

A field survey took place in summer 2016 in the Lower Danube, where two sampling sites were selected according to their invasion history (*sensu* Pârvulescu et al., 2015). The first site (S1) was selected within the old-invaded sector of the river (N 44.804195; E 21.280287) and the second site (S2) within the invasion front (N 44.717095; E 22.405827), where the SCC is currently replacing the native NCC. Crayfish species were caught in the littoral area with bait-traps (61 × 31.5 × 25 cm, pirate type, with double entrance), left over night. All crayfish (site S_1: $n = 17$ (NCC) and 16 (SCC); site S_2: $n = 16$ (NCC) and 19 (SCC)) were captured and euthanized according to animal welfare regulations and measured for total length using a digital calliper of 0.01 mm accuracy (Stanley Black and Decker, New Britain, Connecticut, USA). For each species, total length of individuals (NCC S1: 106.68 ± 0.32 mm and NCC S2: 105.79 ± 0.48 mm; SCC S1: 92.56 ± 0.38 and S2: 92.20 ± 0.70 mm) did not differ significantly between sites (Mann–Whitney tests: $P > 0.05$ for both species of crayfish).

Stable isotope analysis (SIA)

For SIA, fresh samples of abdominal tissues were collected from all captured crayfish (see above). Putative food resources ($n = 3$ for each type) were collected with a pond net as well as by hand and identified in the field. The sampled food resources were then categorised into five groups according to isotopic and taxonomic similarity: (1) mud, (2) leaves (Salix sp.), (3) aquatic primary producers (i.e. macrophytes and filamentous algae), (4) invertebrates (i.e. molluscs and arthropods) and (5) fish (Gobiidae). All invertebrates were left in plastic petri dishes alive for 24 h to clear their gut contents before preparation. Prior to further preparation, molluscs' shells and fish guts were removed. Samples were dried in an oven (60°C, 48 h), homogenised and analysed for stable carbon and nitrogen isotope signatures using a Flash 2000 HT elemental analyser (which also provided the carbon and nitrogen content of samples) coupled via a ConFlo IV interface to a Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). The reported stable carbon and nitrogen isotope values are expressed as δ (‰) relative to the international reference standards Vienna PeeDee Belemnite and atmospheric nitrogen, respectively. An internal reference material (i.e. casein) was measured in duplicate every ten samples revealing a precision (± 1 SD) of $\leq 0.06‰$ for both $\delta^{13}C$ and $\delta^{15}N$.

We estimated the relative contribution (%) of each resource to the diet of each individual crayfish using the Bayesian mixing model provided by the R package SIAR (version 4.2.1.; Parnell et al., 2013; R Development Core Team, 2017). Fractionation factors between resources and crayfish were calculated according to Jackson et al. (2017). Isotopic bi-plots of δ^{13}C and δ^{15}N for crayfish and their putative resources indicated that mud was likely not assimilated by native or invasive crayfish (i.e. low values for both isotopes employed) and was thus removed from further analysis. Therefore, the potential food sources used in the SIAR model were leaves, aquatic primary producers, invertebrates and fish. Trophic position (TP) was calculated according to Olsson et al. (2009):

$$TP = 2 + \left(\delta^{15}\mathrm{N_c} - \delta^{15}\mathrm{N_{inv}}\right)/3.8,$$

where $\delta^{15}\mathrm{N_c}$ is the isotopic value of crayfish, $\delta^{15}\mathrm{N_{inv}}$ is the isotopic value of primary consumers (average δ^{15}N of invertebrates), 3.8 is the fractionation between trophic levels and 2 is the trophic position of the baseline organism.

An omnivory index was calculated according to Jackson et al. (2017): this metric represents an indicator of the diversity of trophic levels consumed by crayfish and, along with their trophic position, gives a more comprehensive image of the role played by a top consumer in a local food web (Christensen & Walters, 2004). δ^{13}C values were converted to a corrected carbon isotope ratio ($\delta^{13}\mathrm{C_{corr}}$), adjusted for between-population variation using the following equation:

$$\delta^{13}\mathrm{C_{corr}} = \left(\delta^{13}\mathrm{C_c} - \delta^{13}\mathrm{C_{litter}}\right) / \left(\delta^{13}\mathrm{C_{primary\ producers}} - \delta^{13}\mathrm{C_{litter}}\right),$$

where $\delta^{13}\mathrm{C_c}$ is the carbon isotope values of crayfish, and $\delta^{13}\mathrm{C_{litter}}$ and $\delta^{13}\mathrm{C_{primaryproducers}}$ are the mean values for leaf litter and primary producers in each site.

Corrected isotopic values were also used to calculate the isotopic niche width of each population using SIBER in the SIAR package (i.e. trophic niche, *sensu* Jackson et al., 2012). We estimated niche widths of both species of crayfish for each site by calculating the standard ellipse area ($\mathrm{SEA_B}$). The calculated $\mathrm{SEA_B}$ contains ca. 40% of the data and provides an estimate of niche area that is less sensitive to differences in sample size (Syväranta et al., 2013). They allow for statistical comparisons of isotope niche widths among populations (Jackson et al., 2011). They were used afterwards to calculate the degree of isotopic niche overlap, representing a quantitative measure of dietary similarity between populations (Guzzo et al., 2013).

The elemental imbalance (EI) of the carbon-to-nitrogen molar ratio (C:N; calculated according to Lauridsen et al. 2012) between crayfish and their food sources was derived from the formula of Cross et al. (2003), by employing the percentage of various food items assimilated in their biomass and C:N. However, the classification of crayfish in a fixed functional feeding guild (*sensu* Merritt & Cummins, 1996) has the disadvantage of allocating them an a priori established diet (i.e. leaves), which does not reflect the omnivorous habit of crayfish (Olsson et al., 2008). Therefore, the EI between both species of crayfish and their food items was calculated as follows:

$$EI = \ln\left(\sum_{i=1}^{n} \frac{\%\mathrm{Resource}_i \times \mathrm{C{:}N}_i}{\mathrm{C{:}N}_c}\right),$$

where $\%\mathrm{Resource}_i$ represents the estimated mean contribution (%) of each resource to the diet according to the SIAR model output for food type i, $\mathrm{C{:}N}_i$ represents the C:N of the same food type, $\mathrm{C{:}N}_c$ represents the mean C:N of crayfish and n the total number of resources as indicated by the model output.

Gut content analyses (GCA)

GCA was performed on the same animals as used for SIA: the tissues remaining after removal of material for SIA were preserved in 4% formaldehyde before later dissection and removal of foreguts. Foregut contents were distributed evenly across a petri dish, following Whitledge & Rabeni (1997). Gut contents were viewed under a dissecting microscope at × 20 magnification and the percentage of the total area of the dish covered by leaves, invertebrates, primary producers (filamentous algae and macrophytes) and inorganic material (i.e. mud) was estimated.

The omnivory index and the EI for C:N of crayfish were calculated as described above, whereas the trophic position was calculated according to Vander-Zanden et al. (1997):

$$\mathrm{TP}_a = \Sigma(V_i \times T_i) + 1,$$

where TP_a is the trophic position of the ath crayfish, V_i is the percentage contribution of the ith prey item and T_i is the trophic position of the ith food item.

Statistical analyses

Trophic niche width and overlap were estimated using the Levins index (Levins, 1968) and the Pianka index (Pianka, 1973), respectively. We tested for significance of overall niche overlap by comparing observed values with those obtained by randomising the original matrices (5000 iterations), using the algorithm ra3 in the EcoSimR package 0.1.0 (Gotelli et al., 2015; R Core Team, 2017).

The percentages of various food items obtained from both GCA and SIA were compared using a two-way ANOVA, with sites, species and their interaction as fixed factors. Prior to testing, an aligned rank transformation (ART) was applied to the percentages of food items. Alignment makes the rank transform procedure more robust when parametric assumptions are violated (Higgins & Tashtoush, 1994; Richter, 1999; Brunner & Puri, 2001). Data were aligned by stripping the marginal means of each effect from the response variables so that all effects were removed except the one for which alignment was applied (Wobbrock et al., 2011). The aligned response was ranked and followed by two-way ANOVA tests with interactions conducted on each factor separately, therefore resulting in separate tests to obtain the F-statistics for sites, species and their interactions. The significance of differences in trophic position, omnivory index and EI for C:N between species and working methods (GCA and SIA) were tested using two-way ANOVA with a priori ART transformation. Calculations for these tests were computed in ARTool package in R, followed by post hoc pairwise comparison of levels within single factors (Wobbrock et al., 2011). In order to comply with the principle of parsimony (Burnham & Anderson, 2001), we performed model simplification and deleted non-significant interactions.

Results

For both crayfish species, the percentage of resource items calculated using GCA did not significantly differ between the two sites, except for the proportion of invertebrate which were more ingested in site S2 (Table 1; Fig. 1A, B). Species had a significant effect on all resource items ingested (Table 1). Specifically, the percentage of leaves, primary producers and mud were significantly higher in NCC foreguts compared to SCC, while the opposite pattern was found for invertebrates (Fig. 1A, B). The SIAR output revealed that both species of crayfish assimilated fish (Fam. Gobiidae), a food item not detected with the GCA approach (Fig. 1C, D). The interaction between Species × Sites was significant for all the assimilated resource items, except the percentage of leaves (Table 1). Specifically, in site S1, the percentages of primary producers and invertebrates were higher in NCC, while the percentage of fish was higher in SCC compared to NCC (Fig. 1C). The percentages of each resource item did not differ significantly between crayfish species in site S2 (Fig. 1D).

Table 1

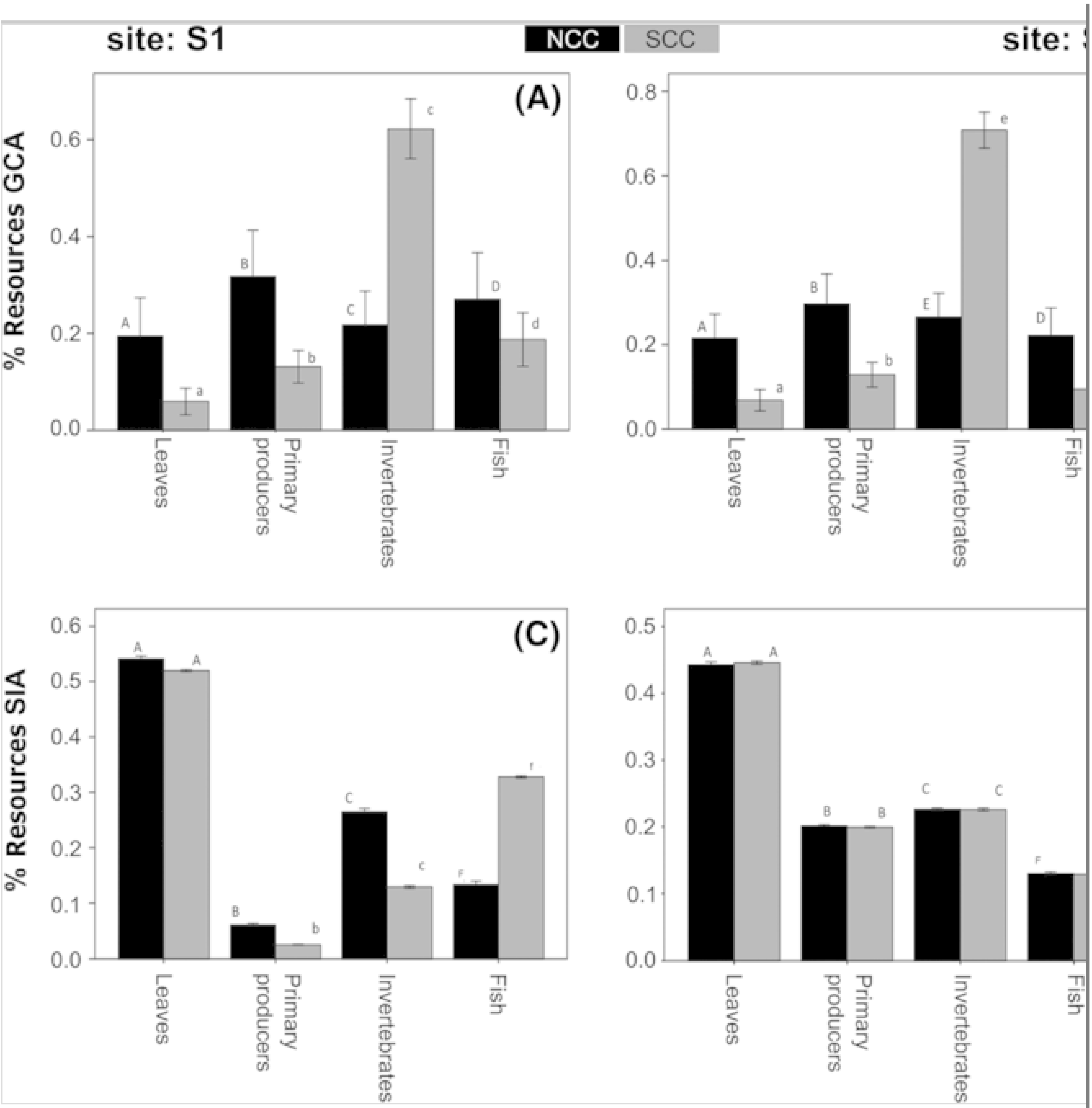
F values of two-way ANOVA models on percentages of food items based on GCA and SIA, for both species of crayfish and sampling sites

Method	Source	Leaves	Primary producers	Invertebrates	Mud (GCA)/Gobiidae (SIA)
GCA	Sites	0.01	0.12	3.57*	1.43
	Species	7.56*	8.07***	54.27***	4.3*
SIA	Sites	209.9***	219.1***	91.21***	213.5***
	Species	7.9	16.08***	151.6***	175.1***
	Sites × species	–	62.28**	172.1***	275.2***

– indicate the removal of interactions between species and sites as a result of model simplification. **P* < 0.05; ***P* < 0.01; ****P* < 0.001

Fig. 1

Mean (± SE) percentage of various food items in the diet of narrow-clawed crayfish (NCC) and spiny-cheek crayfish (SCC) in sites S1 (**A**, **C**) and S2 (**B**, **D**) based on GCA (**A**, **B**) and SIA (**C**, **D**). *NCC* narrow-clawed crayfish (black), *SCC* spiny-cheek crayfish (grey). Significant differences between species for a certain food item within each sampling site are represented by asterisk. **P* < 0.05, ***P* < 0.01, ****P* < 0.001



At site S1, trophic position was significantly higher for SCC compared with NCC for both working methods (Fig. 2A, B, Table 2). Overall, trophic position did not differ between working methods in site S1, but was significantly higher when using GCA compared with SIA for NCC in site S2 (hence the significant interaction between Method × Species, see Table 2). Omnivory index was significantly higher for GCA compared with SIA at both sites (Fig. 2C, D, Table 2). At site S1, omnivory index was significantly higher for SCC compared with NCC, for both working methods, whereas at site S2 the opposite was observed (Fig. 2C, D, Table 2). EI for C:N was significantly higher for GCA compared with SIA at both sites, but did not differ between species (Fig. 2E, F, Table 2).

Fig. 2

Mean (± SE) trophic position (**A**, **B**), omnivory index (**C**, **D**) and elemental imbalance (EI) for C:N (**E**, **F**) in sites S1 (**A**, **C**, **E**) and S2 (**B**, **D**, **F**) for both narrow-clawed crayfish (NCC) and spiny-cheek crayfish (SCC). Black and grey symbolises GCA and SIA, respectively. Significant differences between working methods for trophic metrics within each sampling site are represented by asterisk. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$

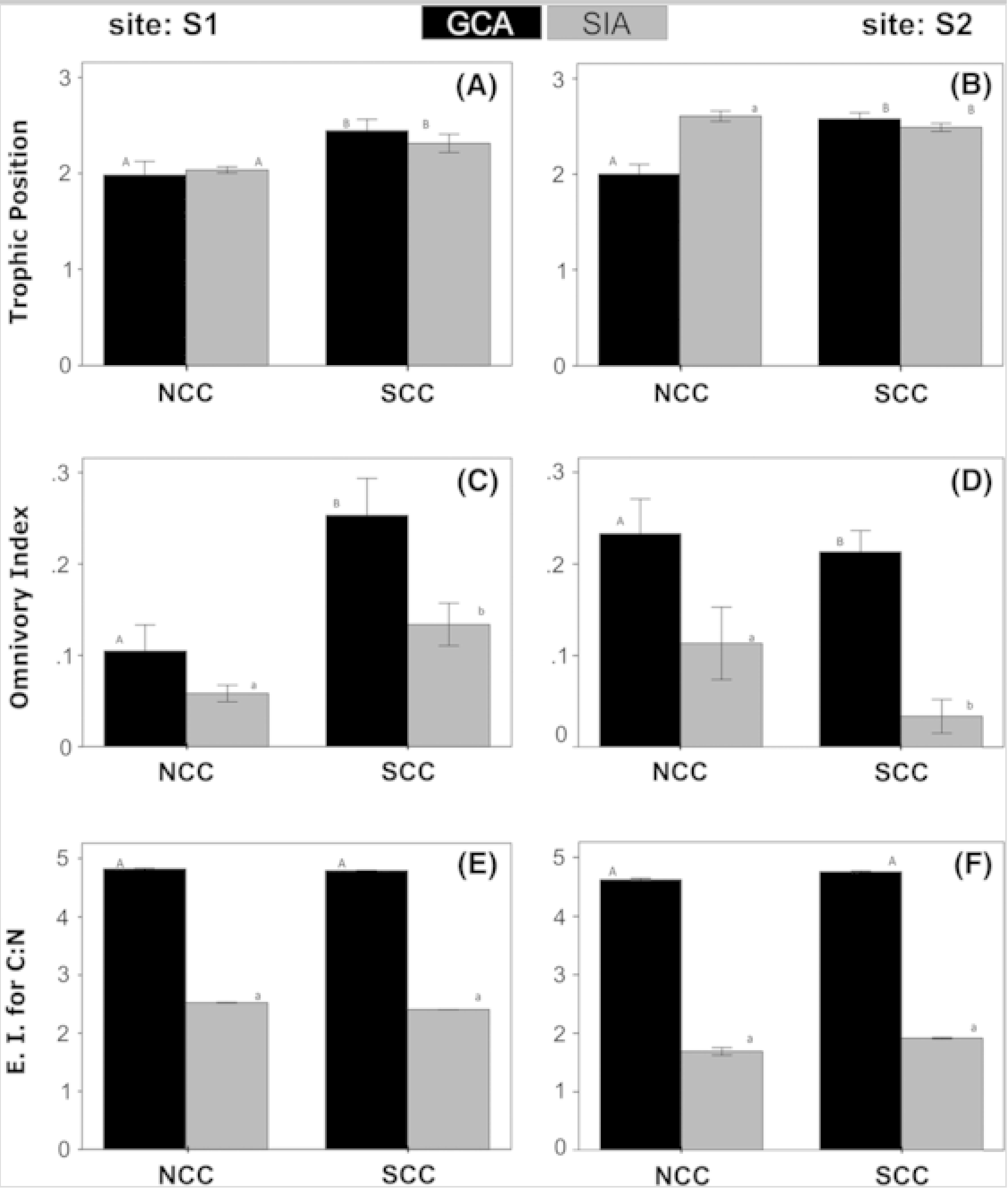


Table 2

F values of two-way ANOVA models for trophic position, omnivory index and elemental imbalance for C:N, for both species of crayfish and methods (GCA and SIA) and their interactions, for sampling sites S_1 and S_2

Sampling site	Source	Trophic position	Omnivory index	Elemental imbalance

site		position	index	imbalance
S1	Method	0.11	8.8***	135.29***
S1	Species	12.35***	16.57***	4.3
S2	Method	15.4***	28.63***	253.4***
S2	Species	18.6*	24.16***	3.62
S2	Method × species	37.01***	–	–

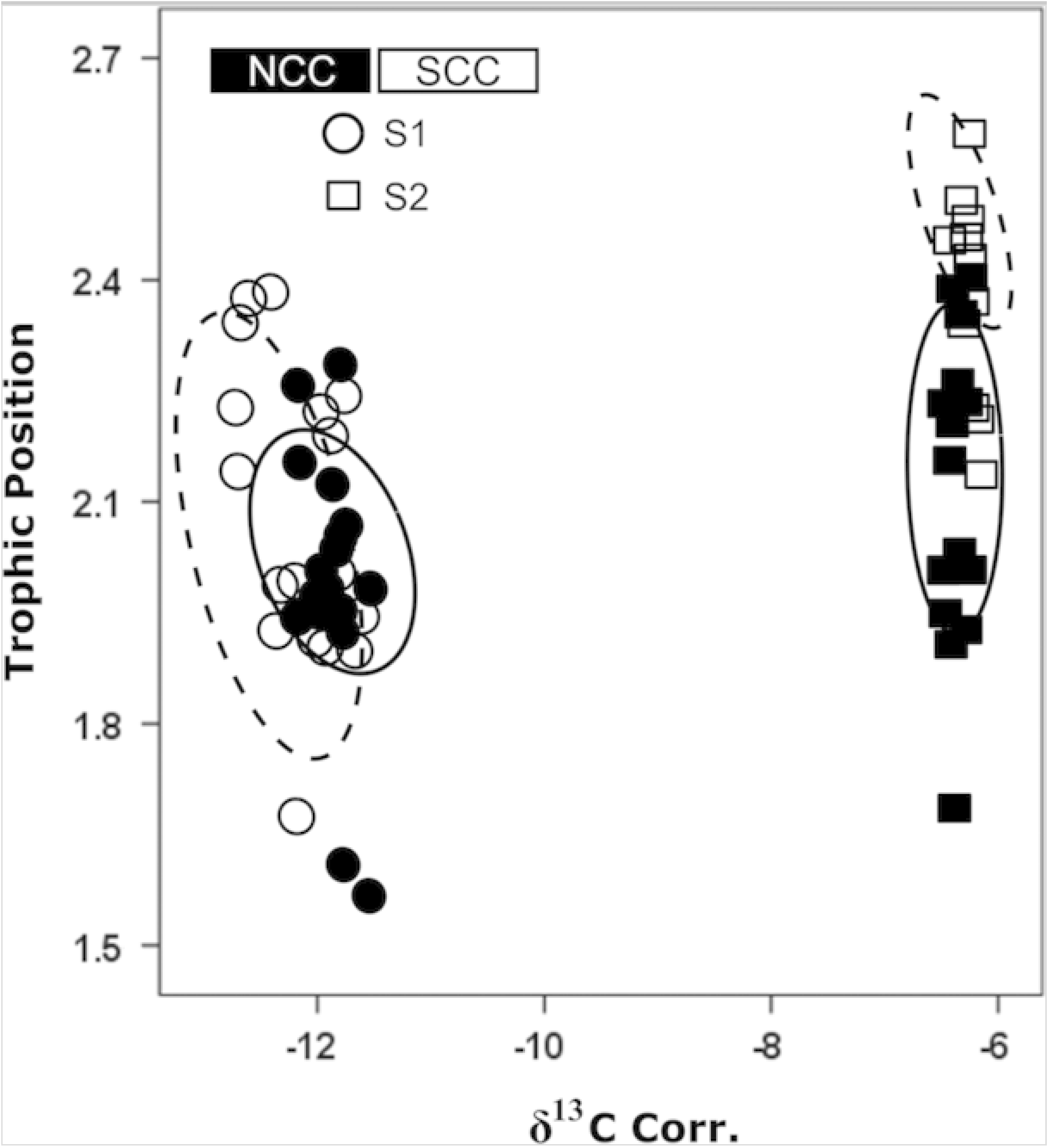
– indicate the removal of interactions between working methods (GCA and SIA) and species (NCC and SCC) as a result of model simplification

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Based on SIA, the trophic niche width of SCC (0.015‰^{2}) in site S1 was significantly smaller compared with that of NCC (0.04‰^{2}) (Fig. 3; $P < 0.001$ pairwise comparison). However, at site S2 the trophic niche width of SCC was twice as large as for NCC (0.19‰^{2} compared to 0.08‰^{2}, Fig. 3; $P < 0.001$ pairwise comparison). The trophic niche of both species overlapped more at site S2 (23.3%) than at site S1 (6%). According to GCA, NCC had a wider niche width than SCC at both sites S1 (3.93 and 2.25) and S2 (3.9 and 1.87). Niche overlaps obtained from GCA were similar at both sites (site S1—69% and site S2—71.4%) and diet similarity seemed to be non-random according to comparisons with simulated data ($P < 0.05$, package EcoSimR).

Fig. 3

Trophic niche width expressed as SEA_b for both narrow-clawed crayfish (NCC, continuous lines) and spiny-cheek crayfish (SCC, dashed lines) for sites S1 and S2



Discussion

SIA and GCA provided similar estimates of trophic position for both species at site S1, whereas at site S2 the former approach indicated a higher trophic position for the native crayfish compared with the latter method (Fig. 2A, B). Previous studies on fish have revealed similar trophic positions using both SIA and GCA (Vander-Zanden et al., 1997; Jones & Waldron, 2003; Franssen & Gido, 2006), whereas others have found differences between methods (Rybczynski et al., 2008; Zambrano et al., 2010). These different results for the native crayfish at sampling site S2 may be attributable to differences in assimilation of ingested food items (Magoulick & Piercey, 2016). While omnivorous crayfish may consume large quantities of basal resources, nitrogen may be obtained mainly from infrequently consumed higher quality resources, such as invertebrates or fish (Roth et al., 2006; Stenroth et al., 2006). This may explain the discrepancy between SIA and GCA noticed for trophic position, omnivory index and EI for C:N (Fig. 2C–F). The significant differences in these indices derived from both working methods may be explained by the predominance of basal resources with high C:N in crayfish foreguts and higher assimilation efficiency for animal tissues with low C:N obtained from higher trophic levels (Cross et al., 2003; Christensen & Walters, 2004; Lauridsen et al., 2012).

Furthermore, GCA and SIA provided differing insights regarding trophic niche width and degree of niche overlap. Outputs from SIA revealed that trophic niche overlap between species in site S1 (6%) was roughly four times smaller than that in site S2 (23%), suggesting stronger interspecific competition for resources in the recently invaded area (Guzzo et al., 2013). GCA, in contrast, indicated a high degree of niche overlap and thus competition at both sites S1 (69%) and S2 (71.4%). Therefore, SIA may indicate resource partitioning with regard to assimilated food items in site S1 (see also SIAR output, Fig. 1C, D). GCA-based niches provide strong evidence that the two species of crayfish compete in terms of diet (Fig. 1A, B). However, the percentages of consumed dietary items corroborate the SIA-based findings that diets differed between the two crayfish species and may suggest that niche estimates based on GCA should be interpreted with caution. The native species ingested significantly more primary consumers, mud and leaves, whereas the invasive crayfish consumed relatively more invertebrates (Fig. 1A, B). This latter finding led to a narrower trophic niche for the invasive crayfish compared with the invasive species at both sites based on GCA. These differences in the items consumed could be related to the different reproductive strategies of the species (*r*- vs *K*-strategists) and to a foraging strategy by the invasive species that maximises reproductive investment as well as growth and hence the likelihood of colonisation success (Phillips & Shine, 2006; Therry et al., 2014). Within the invasion front of the lower Danube, both the signal crayfish [*Pacifastacus leniusculus* (Dana, 1852)] in Croatia and female spiny-cheek crayfish in Romania (both *r*-strategists) displayed greater reproductive success compared with their counterparts (*K*-strategists) from old-invaded-areas, with the former species also being in better condition and energetic status (Pârvulescu et al., 2015; Rebrina et al., 2015).

In the recently invaded site, S2, the trophic niches of both species based on SIA overlapped four times as much as at the long-term invaded sector, S1 (Fig. 3). Strong niche overlaps between native and invasive crayfish species have been found before (Olsson et al., 2009; Ercoli et al., 2014); however, reported changes in the relative dependence on certain food items caused by invasion history for crayfish or other taxa are rare (Vander-Zanden et al., 1999; Beatty, 2006;

Jackson & Britton, 2014), despite being predicted by the principle of competitive exclusion (Bøhn et al., 2008). The prolonged coexistence in the old-invaded site could potentially be based on the species avoiding direct competition by minimising the level of trophic niche overlap through resource partitioning (Fig. 3). While the ingested food items (estimated via GCA) did not differ considerably between sites for both species (Fig. 1A, B), this was clearly the case for assimilated food items (Fig. 1C, D). Moreover, the differences in the relative dependence on the available resources were negligible in the short-term invasion site (Fig. 1D), whereas they were obvious in the long-term invaded sector, notably a larger dependence of NCC on invertebrates (despite the lower dependence indicated by GCA) and a larger reliance of SCC on fish (Fig. 1C). While underlying mechanisms need to be addressed in future research, it is possible that this resource partitioning represents the main factor that has enabled the coexistence of these two species of crayfish at this site for more than a decade, a phenomenon which otherwise is very rare in the Lower Danube (Pârvulescu et al., 2015).

AQ1

Contrasting theories suggest that competition can either decrease population niche width by decreasing the range of resources available to consumers (Jackson et al., 2012) or increase population niche width as individuals consume alternative prey items to maintain their nutritional requirements (Svanbäck & Bolnick, 2006). In fact, both potential diet shifts are forecast by the principle of competitive exclusion, with differing outcomes for the species involved (Bøhn et al., 2008). The observed differences in niche widths between old and recently invaded sites from this study are in line with these theoretical predictions. Trophic position and omnivory were significantly higher for the invasive crayfish compared with the native species in the old-invaded site (Fig. 2A–D), largely due to a higher assimilation of fish by the invasive species (Fig. 1C, D). The differences in the niche widths of both species of crayfish between the old-invaded site and the invasion front demonstrate wide plasticity in diet and may explain the success of biological invasions of this group of invertebrates in inland waters (Olsson et al., 2009) but, equally, the adaptability of the native species to enable long-term coexistence with a superior competitor (Závorka et al., 2018).

Publisher's Note

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