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Research Article

Invasive signal crayfish and native noble crayfish show trophic niche shrinkage in sympatry

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Abstract

Studying the coexistence of native species and invasive species with similar functional traits, habitat usage, and feeding habits is crucial for understanding the dynamics of invasion and ecological changes in the invaded ecosystem. Due to competitive exclusion and often also dissemination of crayfish plague pathogen (*Aphanomyces astaci*, Schikora), North American crayfish represent a major threat to European native crayfish. Their co-occurrence is often only temporary, making studies investigating trophic ecology of native and non-native crayfish species rare. In this study, trophic niche and feeding ecology of European native noble crayfish *Astacus astacus* (Linnaeus, 1758) and North American signal crayfish *Pacifastacus leniusculus* (Dana, 1852) were compared between their sympatric and allopatric sites, in Křesánovský brook (Czech Republic), using carbon and nitrogen stable isotopes analysis. The results indicated a substantial change of trophic niche and diet of noble crayfish between allopatry and sympatry. In allopatry, both juvenile and adult noble crayfish exhibited a wider trophic niche width compared to juvenile and adult signal crayfish. However, in sympatry, where adult and juvenile noble crayfish coexisted with signal crayfish, their trophic niche width significantly narrowed and their diet shifted towards a more plant-based one. High degree of trophic niche overlap was observed between adults and juveniles in both species, particularly in sympatry rather than in allopatry. The substantial trophic niche overlap and dietary similarity between native noble crayfish and invasive signal crayfish in sympatry, make their long term coexistence on the invaded site unlikely.

Key words: Allopatry, coexistence, diet, invasive species, native species, niche ecology, stable isotopes

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Introduction

Native and invasive species coexistence is a pressing issue in species invasions as new species spreading beyond their natural range usually results in competition with native species. It is often claimed that invasive species are superior competitors over native species with the ability to displace them (Pyšek et al. 2020; Somogyi et al. 2023). However, despite increasing interest in the ecological effects posed by invasive species on native communities, the mechanisms that favour an invasive species to successfully establish in a new ecosystem and coexist with native species are complex and often controversial (Godoy 2019). The success of an introduced species largely depends on its functional traits together with combination of environmental factors and presence of specific biota in given ecosystem (Correia 2002). One of the features that successful invaders typically show, is the presence of a wide trophic niche, a common trait among generalist and omnivorous species, that enables them to exploit a wider range of resources e.g. habitat and food sources, compared to natives (Olsson et al. 2009; Ercoli et al. 2014; Modesto et al. 2021).

There are numerous reports of the reduction and local extinctions of native species after the introduction of new species. Displacement mechanisms by which invaders affect native species can be competition (Hill and Lodge 1994; Westman et al. 2002; Rebrina et al. 2015), reproductive interference (Perry et al. 2001) and transmission of pathogens (Westman et al. 2002; Vilcinskas 2015). However, occasionally native species can survive and coexist with invasive counterparts affecting resources competition (Piscart et al. 2011; Altieri and Irving 2017; Pacioglu et al. 2020; Balzani et al. 2021). Usually when two ecologically similar species occupy the same area, competition can either decrease trophic niche width by decreasing the range of resources used by consumers, or increase it as individuals are forced to consume alternative food sources (Svanbäck and Bolnick 2007; Jackson et al. 2012; Jackson et al. 2016; Copp et al. 2017). However, depending on the level of the total resource exploitation and characteristics of the receiving ecosystem, invasive species can affect the structure of the communities and food webs, leading to unexpected outcomes hindering impact assessment (Jackson et al. 2016; Larson et al. 2017).

Invasive crayfish are becoming more prevalent in Europe (Kouba et al. 2014; Weiperth et al. 2020), which leads to increased encounters and competition with native crayfish, leading to collapses of native populations due to competition for resources or spread of crayfish plague, a severe disease spread by often chronically infected North American crayfish species (Jussila et al. 2021). Previous studies indicate that invasive signal crayfish (*Pacifastacus leniusculus*) may have a wider trophic niche at the species level than native noble crayfish (*Astacus astacus*) living in allopatry, although individual populations have been found to occupy rather similar niches (Olsson et al. 2009; Ercoli et al. 2014), likely due to a wider food sources exploitation, and to different habitat use (Ercoli et al. 2015). Recently Pacioglu et al. (2019) reported coexistence of an invasive spiny-cheek crayfish (*Faxonius limosus*) and native narrow-clawed crayfish (*Pontastacus leptodactylus*) in the lower Danube River, claiming that prolonged competition between species induced resources partitioning between species, potentially making their coexistence possible in the future. Veselý et al. (2021) found that trophic niches of two sympatric invasive crayfish shifted from a full overlapping to a more distinct trophic niche in the presence of a third invasive crayfish species. Furthermore, recent studies have indicated an increase in crayfish resistance, or decreased virulence of crayfish plague, in native European crayfish (Ungureanu et al. 2020; Francesconi et al. 2021; Jussila et al. 2021) which may increase the number of competition-coexistence in the future. However, studies on ecological interactions between sympatric native and invasive crayfish populations are scarce, and more investigations are needed to better understand how and at what degree the ecological behaviour of invasive and native crayfish change when they come to leave in sympatry.

The main aim of our study was to investigate trophic niche and food source use of invasive signal crayfish and native noble crayfish in allopatry, where crayfish species were alone, and in sympatry, where were together, in a local brook. Based on earlier studies (Olsson et al. 2009; Ercoli et al. 2014), we hypothesized that (1) the two crayfish species exploit food sources similarly, and their trophic niches would be wider, with more overlap when in allopatry; (2) in sympatry, the increase in intra- and interspecific competition causes changes in diet, involving the use of different food sources. This results in a lower trophic niche width and partitioning, with low overlap.

Methods

Study area

The study was carried out in September 2018, in Křesánovský brook, Czech Republic. Křesánovský brook is located in the foothills of Šumava mountains near the city of Vimperk (Fig. 1). Large males and females of signal crayfish together with younger cohorts were recorded for the first time in the studied brook in 2015 showing an established population. Size of large crayfish found and local fishermen information suggested that signal crayfish could have been introduced in the brook between 2006 and 2009.

Křesánoský brook is shallow with a maximum depth of 0.6 m (mean depth 0.15 m) and a width ranging from 1.2 m up to 2.5 m in the widest part. The brook is composed of relatively cold water even through the summer season when temperatures usually do not exceed 16 °C (15 ± 1.1 °C). The bottom is covered by pebbles, stones, leaves of deciduous trees, and dead wood, providing many possible shelters for crayfish similar to the burrows in clay-sandy banks at many parts of the brook. Previous fieldwork and local fisherman confirmed the absence of fish in the brook.

The study site can be divided into three sections: allopatric noble crayfish site (only noble crayfish present) located upstream (49°3.90433'N, 13°45.12347'E), sympatric site (both noble and signal crayfish present) (49°3.71333'N, 13°45.30298'E), and allopatric signal crayfish site (only signal crayfish present) (49°3.53700'N, 13°45.65420'E), all of which are separated by small weir and a part of piped stream (approximately 40 m long) (Fig. 1). In general, all sections are fairly similar to each other in terms of habitat type, shelter and food source availability, water current, canopy cover and length (approximately 500 m). However, the allopatric noble crayfish site is partly channelised with concrete structures and probably offers less shelter for crayfish than the other two sites. One of the most important factors for the coexistence of invasive signal and native noble crayfish species is the absence of crayfish plague (*Aphanomyces astaci*), which is deadly for the native species (Westman et al. 2002; Jussila et al. 2021). In the studied brook, recent analyses revealed the absence of crayfish plague (Mojžišová et al. 2020).

Crayfish and food sources sampling

Signal crayfish and noble crayfish, along with their potential food sources (macroinvertebrates and detritus), were collected by kick net and by hand in each site of the studied brook. In laboratory, macroinvertebrates were sorted and identified at the lowest taxonomic level, grouped according to their functional feeding group as collectors, filters, scrapers, shredders and predators, and put into glass tubes, as well as allochthonous detritus to be processed for stable isotope analyses later. Crayfish sex was determined, carapace length (mm) and weight (g) were measured and animals were divided into groups: males and females, juveniles (carapace length < 30 mm) and adults (carapace length ≥ 30 mm). Semi-quantitative macroinvertebrate sampling was conducted using a kick-net in each site to provide additional data on macroinvertebrate food source availability.

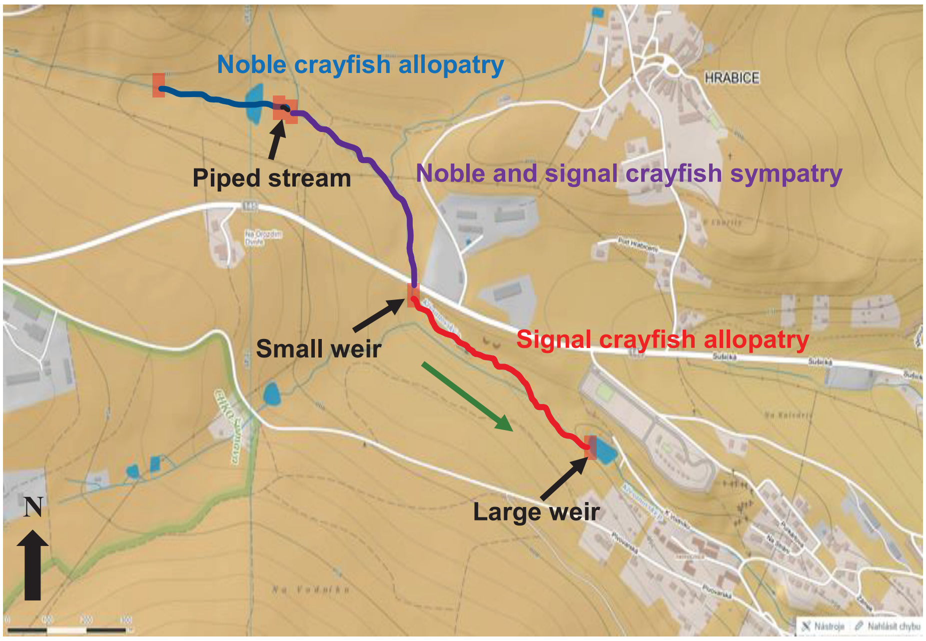

Figure 1. Map of the studied brook indicating different sites in different colours, where crayfish and food sources were sampled. The blue stretch of the brook indicates allopatric noble crayfish site, purple indicates noble and signal crayfish sympatric site, and the red signals crayfish allopatric site. Black arrows indicate weirs, green arrows indicate the flow direction.

Stable Isotopes analyses

All samples were dried in an oven at 60 °C for 48 hours and grinded to a fine homogeneous powder. From animal and plant samples, 0.6 mg and 1.0 mg of materials were weighed respectively into tin caps. All the samples were analysed for carbon and nitrogen stable isotopes with a FlashEA1112 elemental analyser coupled to a Thermo Finnigan DELTAplus Advantage continuous flow isotope ratio mass spectrometer (Thermo Electron Corporation, Waltham, MA, U.S.A.) at Jyväskylä University in Finland. Reference materials used were internal standards of known relationship to the international standards of Vienna Pee Dee, including belemnite for carbon isotopes and atmospheric nitrogen for nitrogen isotopes. Stable isotope ratios are expressed as parts per thousand (‰) delta values relative to the international standards for carbon and nitrogen. White muscle tissue of northern pike *Esox lucius* (Linneaus, 1758) for animal based samples and birch leaves *Betula pendula* for detritus with known isotopic compositions were used as internal working standards to ensure precision of the analyses. One standard sample was run repeatedly after every five samples in each sequence. Standard deviations within reference samples in each sequence were less than 0.1 ‰ for carbon and 0.2 ‰ for nitrogen in pike and in birch leaf samples.

Trophic niche

Crayfish were divided into groups, according to their site of capture and size (juveniles and adults). The trophic niche width of signal crayfish and noble crayfish juveniles and adults, in both allopatry and sympatry were calculated as the Bayesian Standard Ellipse Area (SEA.B; encompassing 95% of the data points) and the corrected standard ellipse area (SEAc; considering 40% of central data points), the latter less sensitive to small sample sizes (Jackson et al. 2011), using the R package SIBER (Stable Isotope Bayesian Ellipses in R) (Jackson et al. 2011). To estimate the degree of trophic niches similarity, the overlap between two ellipses were calculated (Jackson et al. 2019). The overlap is the area, in units of per mil squared (‰^{2}) contained by the shape that lies within the overlapping region, and it is calculated by using the SEAc of each ellipse. Trophic niche overlapping was used to reveal the degree of ecological similarity between adults and juveniles, noble crayfih and signal crayfish populations in allopatry and in sympatry. Trophic niche area of each crayfish group was compared by posterior distribution of paired trophic niches and then calculating their probability of posterior distribution similarity (Jackson et al. 2019). The proportions' range vary from 0, when the ellipses are not overlapping, to 1, when the trophic niches are completely overlapping.

Diet

Collectors, filter feeders and scrapers macroinvertebrate functional groups were grouped to represent one food source (hereafter cfs), due to their similar carbon and nitrogen isotopic values. Proportions of four food sources (shredders, cfs, predator macroinvertebrates and detritus) used by adults and juveniles of both crayfish species living in allopatry and sympatry, were calculated using MixSIAR Bayesian mixing models in R. (Stock and Semmens 2016b; Stock et al. 2018). Crayfish juveniles were also considered as a food source for adults (Veselý et al. 2020). Due to their substantial overlap in carbon and nitrogen stable isotope values showed by MixSIAR model, juvenile crayfish and macroinvertebrate predators sources were pulled together representing one food source (hereafter predator). MixSIAR models were run including the species and age classes, allopatric and sympatric sites as fixed factors selecting residual and process errors (Stock and Semmens 2016a). Fractionation factors for δ^{15}N and δ^{13}C were assumed as 3.23 ± 0.41‰ and 0.47 ± 1.23‰ respectively for macroinvertebrate (Zanden and Rasmussen 2001), while for detritus 2.4 ± 0.42‰ for δ^{15}N and 0.40 ± 0.28‰ for δ^{13}C (McCutchan et al. 2003). The models were run using Markov Chain Monte Carlo (MCMC) parameters of three chains of 300,000 iterations, burn-in phase of 200,000, and thinning of 100. The percentage contributions of food sources to crayfish diets were generated by the models as posterior distributions with 95% credibility intervals, for each size class and site. Gelman-Rubin and Geweke tests were employed for testing convergence and diagnostic statistics of all model results. For the first test all variables must have values < 1.05 and for the second test means of the first and second part of the chain must be the same.

Statistical analyses

One-way ANOVA was used to test for differences in δ^{13}C and δ^{15}N values of food sources between sites. Differences in δ^{13}C and δ^{15}N of signal crayfish and noble crayfish between males and females, juveniles and adults, noble crayfish and signal crayfish allopatric sites and sympatric site, were tested using One-way ANOVA. Significant differences (p-value < 0.05) from ANOVAs were further analysed using post-hoc Tukey-HSD pairwise test for comparisons between sites. Fisher's exact test was used to test for differences in shredders, collectors-filters-scrapers (cfs) and predators' macroinvertebrate abundance distributions between noble crayfish allopatric, signal crayfish allopatric and noble and signal crayfish sympatric sites. Assumptions for normality and homogeneity of variances were tested prior to statistical analyses using Shapiro-Wilk and Levene tests, respectively. All statistical analyses and Bayesian models were performed in R (R Core Team 2022).

Results

In total, 87 crayfish, 38 noble crayfish (16 females and 22 males, 15 adults and 23 juveniles) and 49 signal crayfish (19 females and 30 males, 22 adults and 27 juveniles) were caught and analysed for carbon and nitrogen stable isotopes. Density of signal crayfish in allopatry was higher (> 6 individuals per m^2) than noble crayfish in allopatry (< 1 individual per m^2) and noble crayfish and signal crayfish in sympatry sites (> 2 individuals per m^2).

Abundances of shredders, collectors-filters-scrapers (cfs) and predators macroinvertebrate were not different (p-value = 0.56, p-value = 0.35; p-value = 1) between allopatric noble crayfish, allopatric signal crayfish and sympatric noble and signal crayfish sites. In general, each site was dominated by shredders, collectors, filtrators and scrapers, while predators were less abundant (Suppl. material 1).

No significant differences were found in the carbon and nitrogen isotope values of food sources between the three studied sites (p-value > 0.05) (Suppl. material 2). δ^{13}C values of both crayfish species differed between their allopatric and sympatric sites (p-value = 0.02 for noble crayfish, p-value < 0.001 for signal crayfish) (Fig. 2, Table 1). Noble and signal crayfish muscle tissue δ^{15}N values differ significantly within and between allopatric and sympatric sites (p-value < 0.001) (Fig. 2, Table 1).

Comparisons between adults and juveniles within sites showed significant differences in δ^{13}C values in allopatric noble crayfish (p-value = 0.03) and allopatric signal crayfish (p-value < 0.001) sites, while δ^{15}N values were different only in allopatric signal crayfish (p-value = 0.002) (Fig. 3). However, neither δ^{13}C nor δ^{15}N values of signal crayfish and noble crayfish differed significantly between females and males, in any allopatric nor sympatric sites. While noble crayfish and signal crayfish adults showed significant differences in δ^{13}C values, between allopatric and sympatric sites, juveniles did not exhibit any significant difference among sites (Fig. 4). However, δ^{15}N values of adults and juveniles of noble crayfish and signal crayfish significantly differ between allopatric and sympatric sites (Fig. 4).

Trophic niches

SIBER model results show that the trophic niche width (SEAc) of adult and juvenile noble crayfish and juvenile signal crayfish shrunk when species were in sympatry (Fig. 5, Table 1). Adult signal crayfish did not show a similar change in trophic niche width, but its position shifted from a lower to slightly higher trophic level and from more depleted to more enriched carbon isotope values (Fig. 5). In allopatry, both species indicated a high interspecific overlap in the trophic niches between adults and juveniles (Fig. 5, Table 2). However, there was little intraspecific overlap in trophic niches when populations were in allopatry (Fig. 5, Table 2). In sympatry, there was a clear increase in both inter- and intra-specific overlap of trophic niches for both species (Fig. 5, Table 2), particularly evident in the signal crayfish.

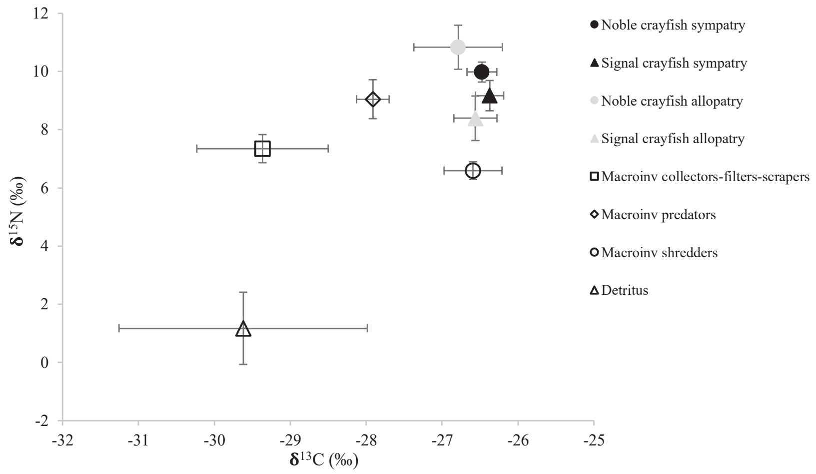

Figure 2. Carbon and nitrogen stable isotopes mean values (± standard deviation) of crayfish groups (noble crayfish and signal crayfish) in allopatry and sympatry and their food sources.

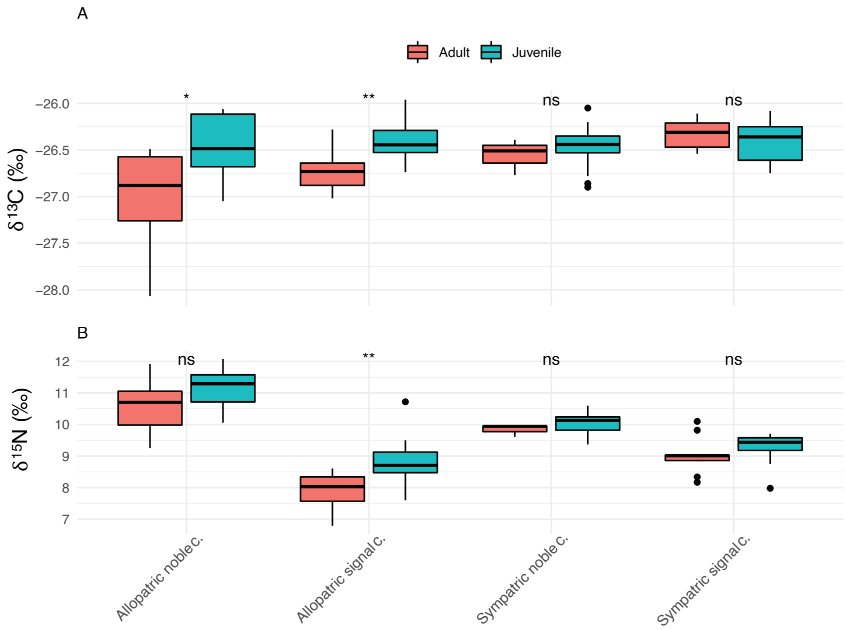

Figure 3. Comparisons of carbon **A** and nitrogen **B** stable isotope values between adults and juveniles within each group of noble and signal crayfish living in allopatry and sympatry. Asterisks (*) (**) and (ns) indicate the significant and not significant difference between the two size classes, respectively.

Table 1. Bayesian standard ellipse area (SEA.B), standard ellipse area corrected (SEAc), carbon and nitrogen stable isotopes mean values (± standard deviation), number of individuals and their length mean values (± standard deviation), divided by group and community.

Community	Group	SEA.B‰^{2}	SEAc‰^{2}	δ^{13}C‰	δ^{15}N‰	N	Carapace length (mm)
Allopatry	Noble crayfish adult	1.09	1.23	-27.04 ± 0.54	10.58 ± 0.74	10	34.99 ± 3.21
	Noble crayfish juvenile	0.78	0.91	-26.48 ± 0.36	11.16 ± 0.63	8	26.20 ± 1.90
	Signal crayfish adult	0.27	0.29	-26.73 ± 0.18	7.94 ± 0.52	13	35.46 ± 2.71
	Signal crayfish juvenile	0.51	0.56	-26.40 ± 0.23	8.82 ± 0.73	14	23.29 ± 1.83
Sympatry	Noble crayfish adult	0.08	0.11	-26.51 ± 0.16	9.83 ± 0.14	5	36.75 ± 4.76
	Noble crayfish juvenile	0.25	0.27	-26.47 ± 0.23	10.02 ± 0.34	15	20.88 ± 4.15
	Signal crayfish adult	0.28	0.32	-26.33 ± 0.15	9.03 ± 0.58	9	42.56 ± 7.86
	Signal crayfish juvenile	0.29	0.32	-26.40 ± 0.21	9.27 ± 0.46	13	18.62 ± 5.18

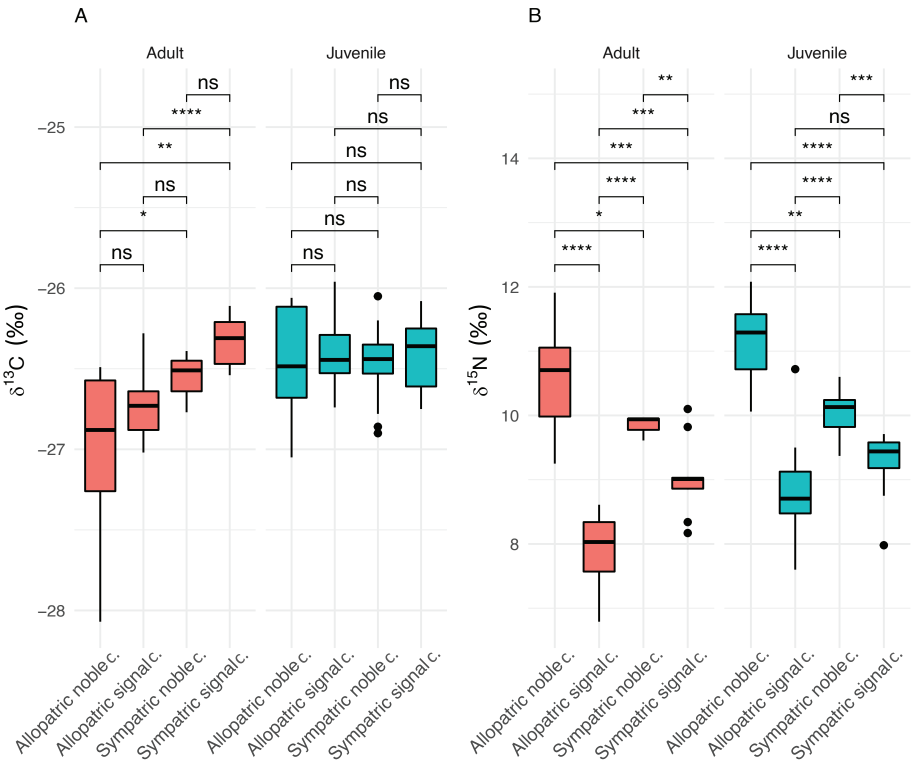

Figure 4. Comparisons of carbon **A** and nitrogen **B** stable isotope values of adults and juveniles between noble and signal crayfish living in allopatry and sympatry. Asterisks (*) (**) (***) and (ns) indicate the significant and not significant difference, respectively.

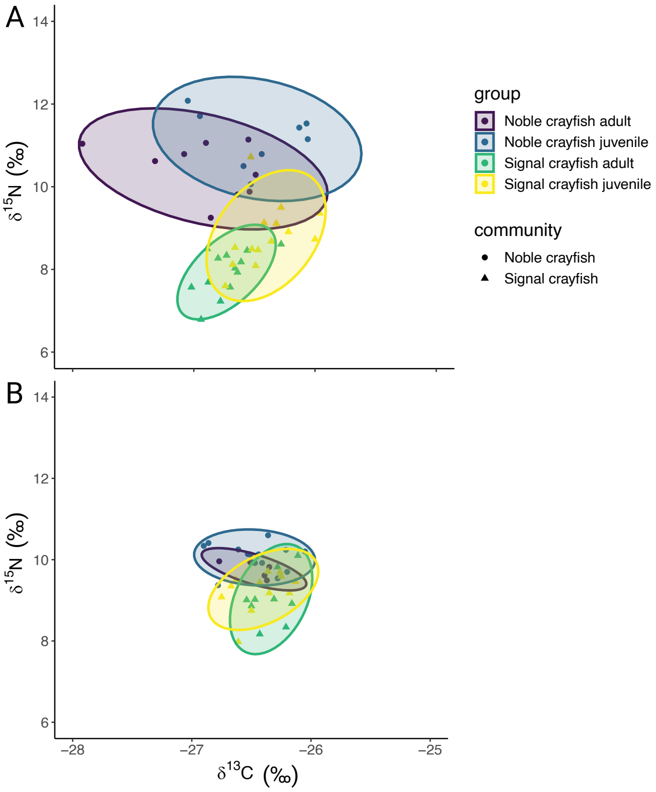

Figure 5. Trophic niche areas, represented by ellipses, of adults and juveniles noble crayfish and signal crayfish in allopatry **A** and sympatry **B**.

Table 2. Proportions of overlapping (%) between each paired group within the two communities.

Community	Group	Overlapping proportions (%)
Allopatry	Noble crayfish adult vs Noble crayfish juvenile	33
	Noble crayfish adult vs Signal crayfish adult	3
	Noble crayfish adult vs Signal crayfish juvenile	20
	Noble crayfish juvenile vs Signal crayfish adult	0
	Noble crayfish juvenile vs Signal crayfish juvenile	12
	Signal crayfish adult vs Signal crayfish juvenile	34
Sympatry	Noble crayfish adult vs Noble crayfish juvenile	37
	Noble crayfish adult vs Signal crayfish adult	19
	Noble crayfish adult vs Signal crayfish juvenile	23
	Noble crayfish juvenile vs Signal crayfish adult	27
	Noble crayfish juvenile vs Signal crayfish juvenile	33
	Signal crayfish adult vs Signal crayfish juvenile	61

Diets

Noble crayfish adults and juveniles used fairly similar proportions of predatory macroinvertebrates in allopatry (47% and 50%) and in sympatry (17% and 19%), while detritus (35% and 38%) and macroinvertebrate shredders (33% and 28%) were used more when in sympatry than in allopatry (Fig. 6A–D, Suppl. material 3). However, signal crayfish adults and juveniles used similar proportions of detritus (42% and 49%) and macroinvertebrates shredder (49% and 39%) in allopatry, while in sympatry, adult and juvenile fed mainly on detritus (60% and 66%) (Fig. 7A–D, Suppl. material 3). However, predatory macroinvertebrates proportions were low in signal crayfish, where allopatric adults and juveniles consumed only 5% and 8% respectively (Fig. 7A, C, Suppl. material 3). Macroinvertebrates cfs source seemed not to be used in the diets of noble or signal crayfish adult and juvenile in allopatry or sympatry (Figs 6A–D, 7A–D, Suppl. material 3).

Discussion

This study indicated a general trophic niche shrinkage of invasive signal crayfish and native noble crayfish when living in sympatry. While only juvenile signal crayfish trophic niche shrink, both adults and juveniles noble crayfish exhibited a remarkable trophic niche shrinkage when in sympatry, decreasing their trophic niche widths, leading to shifts in diets and trophic levels.

Previous studies have indicated that the coexistence of invasive and native species results in trophic niche partitioning, leading to a decrease in niche width and low overlap (Thomson 2004; Jackson et al. 2012; Eloranta et al. 2013; Tran et al. 2015; Balzani et al. 2021) likely due to shifts in diet or habitat. In crayfish, both inter- and intraspecific competition during sympatric coexistence might induce trophic niche partitioning, stemming from differences in use of food sources and/or habitat (Jackson et al. 2014; Jackson and Britton 2014; Pacioglu et al. 2019). Contrary to our first hypotheses, where we expected wider trophic niches and overlap between species in allopatry, our results indicated that trophic niche overlap was higher and niche widths were clearly lower when the species were in sympatry.

In sympatry, crayfish species occupied similar trophic niches which overlapped substantially. A higher crayfish population density in sympatry might have led to

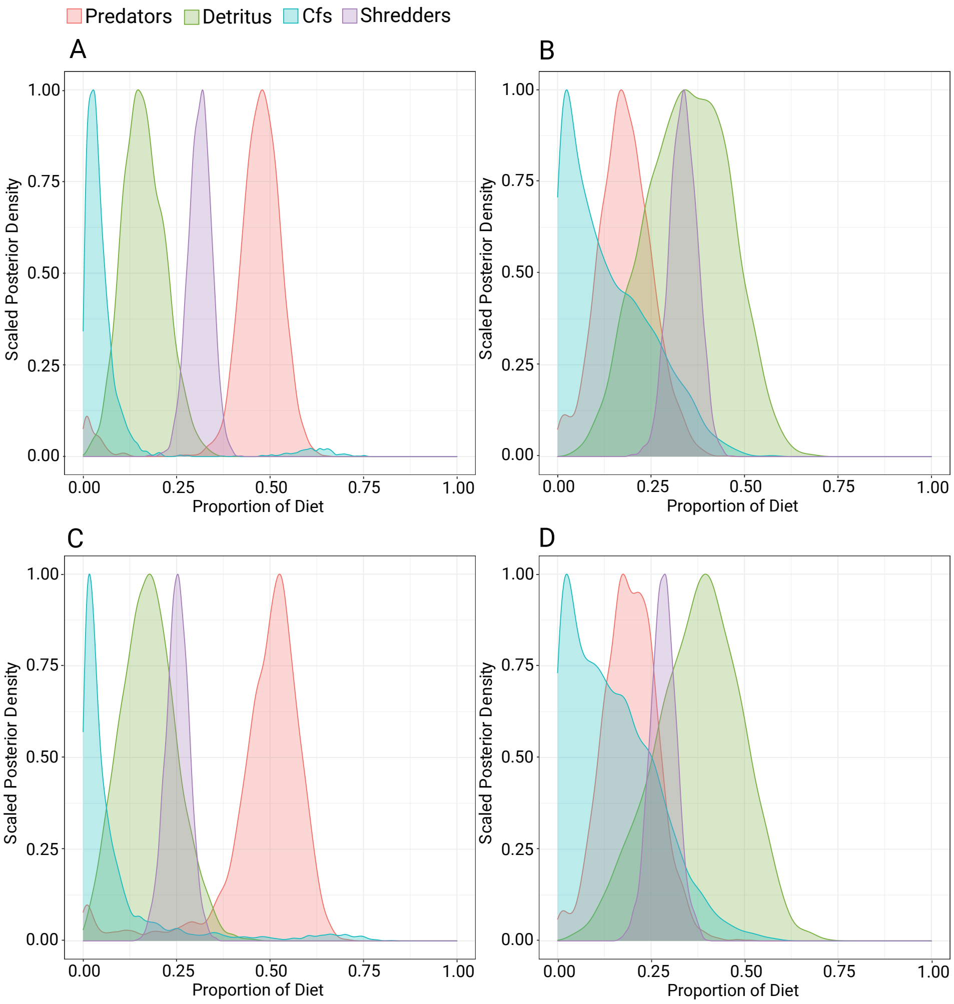

Figure 6. MixSIAR models indicating posterior distribution of food source proportions (predator macroinvertebreates, cfs macroinvertebrates, shredder macroinvertebrates and detritus) used by noble crayfish adults **A** and juveniles **C** living in allopatry, and by noble crayfish adults **B** and juveniles **D** living in sympatry.

increased inter- and intraspecific competition. In our study, sympatric noble crayfish was forced to share habitat and energy sources within a rather restricted habitat, with higher crayfish density, most probably without the possibility of niche partitioning. Larson et al. (2017) investigated the trophic ecology of coexisting invasive crayfish, emphasising the role of the ecosystem on the food web structure and the availability of food sources, subsequently influencing the trophic level of sympatric crayfish species. For instance, Ercoli et al. (2014) found that signal crayfish and noble crayfish trophic niches strongly overlapped and that signal crayfish exhibited a wider trophic niche compared to native noble crayfish, suggesting a wider use of habitat and food resources. Similarly, Olsson et al. (2009) found that signal crayfish occupied a two-fold wider trophic niche than native noble crayfish in Swedish streams. Moreover, they found that high food source availability can also drive a wider trophic niche in crayfish species. Nevertheless, in Ercoli et al. (2014) and Olsson et al. (2009) the comparison of signal crayfish and noble crayfish trophic niches was done between allopatric populations without real coexistence. Our study area is a small brook with a limited amount of habitat, likely the higher density of crayfish in sympatry, along with limited space and food resources sharing, caused decreasing of trophic niche width of noble crayfish and high trophic niche overlap between ecologically rather similar crayfish species.

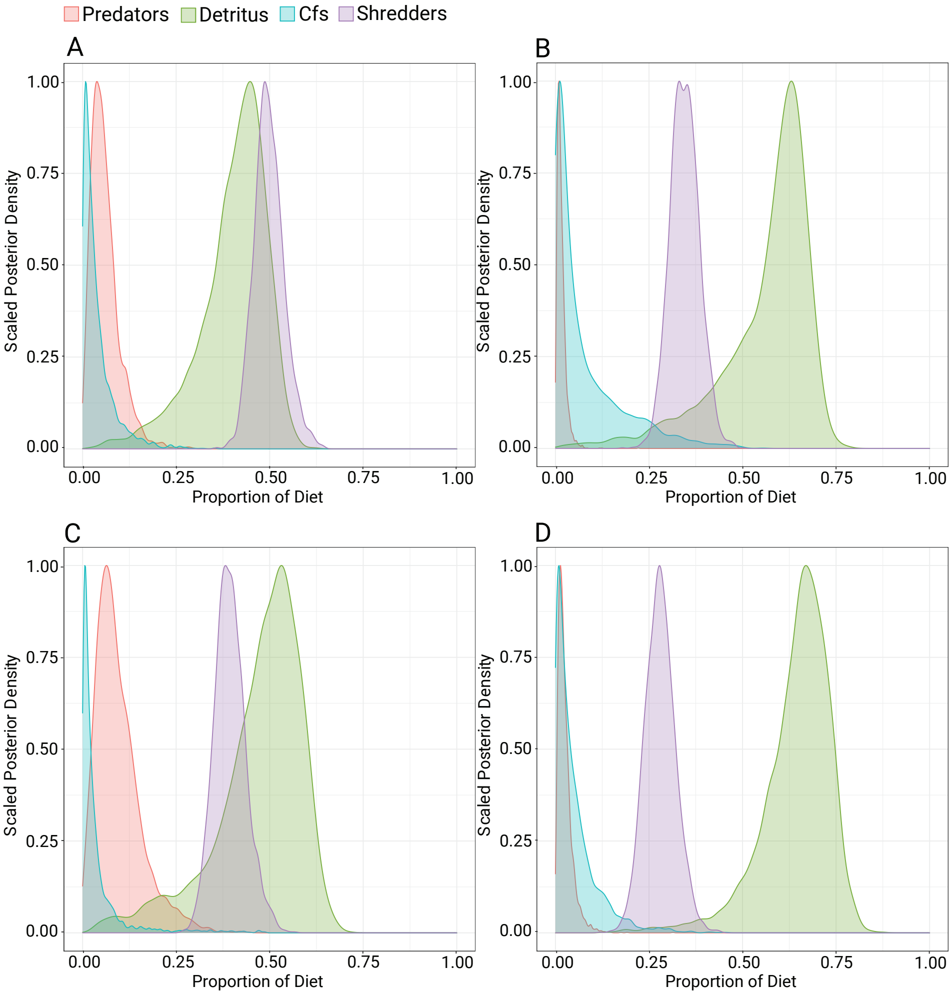

Figure 7. MixSIAR models indicating posterior distribution of food source proportions (predator macroinvertebreates, cfs macroinvertebrates, shredder macroinvertebrates and detritus) used by signal crayfish adults **A** and juveniles **C** living in allopatry, and by signal crayfish adults **B** and juveniels **D** living in sympatry.

Noble crayfish and signal crayfish could have experienced different food source availability between allopatry and sympatry, due to the habitat sharing when in sympatry, which could have influenced their food source use and trophic niches. However, semiquantitative macroinvertebrates data indicated similar macroinvertebrate communities and abundances among the three studies sites (Suppl. material 1), providing similar and abundant food source availability. Yet, riparian habitats and vegetation were similar between sites by visual assessment. These similarities suggest that changes in diets and trophic niches were not driven by differences in food source availability between sites, but rather by the increased competition when crayfish were in sympatry. Ella et al. (2016) highlighted the higher food source consumption generally exhibited by invasive species compared to their native conspecifics. This aspect has also been observed between signal and noble crayfish. In a previous study, Nyström et al. (1999) found that signal crayfish consumed more macroinvertebrate primary consumers and macrophytes than native noble crayfish. Furthermore, results from Hudina et al. (2015) indicated that invasive signal crayfish reduced their aggressive behavior at the invasion front, in sympatry with native conspecific, compared to when the species was in allopatry. In our study, the increased interspecific competition between sympatric invasive and native crayfish species, combined with the high foraging of signal crayfish, likely affected the feeding behavior of native noble crayfish, making the latter less active and changing its predatory behaviour. Ecologically similar invader and native species may highly compete for resources and habitats when in sympatry. Previous studies indicated that intraguild predation (IGP), the interspecific interactions between closely related species that have the ability to prey on each other and on shared preys, can be seen as one of the main drivers in species extinction and replacement (Polis et al. 1989; Holt and Polis 1997). For instance, previous studies on invasive and native Amphipoda species indicate that IGP was the main driver leading to the replacement of native *Gammarus duebeni celticus* by invasive *Gammarus pulex* in nature (Dick et al. 1993, 1999). However, IGP may have direct or indirect effects, such as suppressing competitive conspecifics via direct predation or modifying their ecological behavior, resulting in trophic niche shifts or decreased foraging behaviour (Polis et al. 1989). In our study, results suggest that IGP had indirect effects on native noble crayfish, modifying its trophic niche and diet. The increasing of IGP could also initially increase and then decrease prey density, depending on the strength of the IGP (Chang and Cardinale 2020). In our case, the availability of food sources, such as macroinvertebrate preys, did not differ between allopatric and sympatric sites.

However, the habitat type was slightly different at the noble crayfish site where the brook was more channelised. This may partly explain the observed differences, such as the different foraging behaviour by noble crayfish at this site. Nevertheless, our results suggest that the changes in trophic niche and diet observed in both species were caused by increased competition for resources in a limited habitat rather than the habitat structure itself.

Besides, it is worth noting that interspecific competition and IGP resulting from trophic interactions between invasive and native species can be affected by the presence of disease (Dick et al. 2010). However, in our study, both signal and noble crayfish individuals did not carry diseases like crayfish plague (*Aphanomyces astaci*) or parasites that could have affected our results (Mojžišová et al. 2020).

Our findings showed that trophic niche of adult noble crayfish occupied almost one trophic level higher compared to that of signal crayfish, indicating a different utilisation of energy sources. Larson et al. (2017), in a study conducted in lake ecosystems, interestingly found a higher trophic position of signal crayfish when compared to red swamp crayfish *Procambarus clarkii*, regardless of their occurrence in sympatry or allopatry. However, it is worth noting that in Larson et al. (2017) study, signal crayfish was in its native area while red swamp crayfish was considered the invasive species. The higher trophic level of invasive signal crayfish niche found in Larson et al. (2017), and lower trophic level found in our study, highlight its high trophic behaviour plasticity, which might depend on its native or non native context. Nevertheless, it is important to consider that in Larson et al. (2017) comparisons were made in lake ecosystems with likely more habitats and food sources availability. In a meta-analytical study comparing the trophic niches of invasive fish between native and invaded ecosystems, Comte et al. (2017) highlighted the ability of invasive fishes to shift their trophic niche towards an intermediate trophic position. Moreover, previous studies have suggested that, relying on a diet from a lower trophic level, could make invasive fish more successful in invaded habitats, given the limitless food sources (Gido and Franssen 2007). In our studied brook, signal crayfish might have also exhibited such invasive strategy, owing to its high trophic plasticity.

Trophic niche and diets are known to change according to crayfish sex and size classes (Usio and Townsend 2002; Larson et al. 2010; Veselý et al. 2020). In contrast with results found by Ercoli et al. (2021), in our study, juvenile and adult signal crayfish indicated different carbon and nitrogen isotopic values in allopatry, which shifted juveniles towards more enriched carbon and higher trophic position compared to adults. However, our results indicated that, when in sympatry, both adults and juveniles of noble and signal crayfish underwent changes in trophic positions. Noble crayfish shifted towards lower trophic positions, while signal crayfish towards higher positions changing their diets markedly when in sympatry and suggesting a shift to a more terrestrial-detritus energy source.

Food source use models (MixSIAR) support our trophic niche results, indicating diets of allopatric noble crayfish and signal crayfish generally composed of higher proportions of macroinvertebrate shredders, while in sympatry both species indicated a shift to more detritus-based diets. Predatory macroinvertebrates' use or crayfish cannibalism (predator macroinvertebrate source included also juveniles crayfish) were high in both juvenile and adult noble crayfish living in allopatry. Thus, model results suggest that diets of both size classes of noble crayfish, were protein-based, mostly composed by predator-omnivorous food items. Pacioglu et al. (2019) also found that native crayfish were more carnivorous when they were in allopatry compared to when in sympatry with the invasive crayfish. However, high proportion of detritus and macroinvertebrates shredders fed in sympatry by both size classes and crayfish species, reflected the smaller niche width shifted at lower trophic level, in particular for noble crayfish. The high similarity of diets in sympatric invasive and native crayfish was also found by Pacioglu et al. (2019).

Conclusions

The trophic niche overlap and diet similarity of native noble crayfish and invasive signal crayfish in sympatry, coupled with limited availability of alternative resources and habitats, make their long term coexistence in Křesánovský brook unlikely, thereby posing a threat to noble crayfish (Westman et al. 2002). Although the eradication of invasive species is challenging, efforts to control the further spread of signal crayfish, and the restoration of the native noble crayfish population, could help preserve the native species (Perales et al. 2021). Additional research is needed to address the knowledge gap concerning the ecological interactions and responses of native and invasive species when living together. This might include assessing the specific response of interacting species and making comparisons across different ecosystems over an extended period.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization FE, TR, LV. Investigation FE, TR, MB, AK, MB and LV. Formal Analysis FE and TR. Writing initial draft FE, TR and LV. Revision and approval of the final submission FE, TR, MB, AK, MB and LV.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Semiquantitative macroinvertebrates representing eudominant and dominant species (++), sub- dominant and recedent species (+) and sub-recedent species (-)

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Data type: pdf

Link: https://doi.org/10.3897/neobiota.98.127329.suppl1

Supplementary material 2

Carbon and nitrogen stable isotopes mean values (standard deviation) and number of samples (n) of food sources in each site

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Data type: pdf

Link: https://doi.org/10.3897/neobiota.98.127329.suppl2

Supplementary material 3

Posterior distribution represented by mode (50%) and confidence interval (2.50% and 97.50%), of food sources used by noble crayfish and signal crayfish adults and juveniles in allopatry and sympatry

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