

Research Article

Trophic position and niche overlap of an Asian weatherfish (*Misgurnus bipartitus*), western tubenose goby (*Proterorhinus semilunaris*) and native benthic fish species

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Academic editor: Michal Grabowski

Received: 9 May 2023

Accepted: 31 October 2023

Published: 7 February 2024

Citation: Lemmers P, Olde Wolbers R, Van der Velde G, Leuven RSEW (2024) Trophic position and niche overlap of an Asian weatherfish (*Misgurnus bipartitus*), western tubenose goby (*Proterorhinus semilunaris*) and native benthic fish species. *Aquatic Invasions* 19(1): 85–108. <https://doi.org/10.3391/ai.2024.19.1.116273>

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Abstract

Co-occurring and morphologically similar species have adapted to differential niches to minimize competition. An invasive alien species can occupy an ‘empty niche’ in introduced ranges. Alternatively, the invader may occupy an overlapping niche and compete with native species to a certain degree. In a Western European lowland brook with high nutrient loads, we studied a benthic community of five fish species, including two alien species: an Asian weatherfish (*Misgurnus bipartitus*) and the western tubenose goby (*Proterorhinus semilunaris*). The native species concerned stone loach (*Barbatula barbatula*), spined loach (*Cobitis taenia*), and gudgeon (*Gobio gobio*). Because of the unknown effects of the invaders on native benthic fish species, the trophic position, isotopic niche overlap, and potential food competition among these species were identified using nitrogen and carbon stable isotopes. The trophic levels of the five fish species indicated that they are secondary consumers. The body size of native fish species correlated significantly negatively with their $\delta^{15}\text{N}$ (‰) signature, in contrast with the invaders indicating that the latter are generalists. Significant isotopic niche overlap was observed among all benthic species. The degree of niche overlap of *M. bipartitus* was the highest with *G. gobio* (91.8%). *Proterorhinus semilunaris* showed the highest degree of niche overlap with *B. barbatula* (91.2%). It was notable that the observed niche overlap between the native *B. barbatula* and *C. taenia* was high (99.2%). Overlap between *M. bipartitus* and *P. semilunaris* was low (8.9%), indicating little resource competition between these alien species. Native species showed wider isotopic niches than the invaders. Bayesian mixing models revealed that native and alien species slightly differ in their main diet. The results suggest that the invaders are plastic in their resource use, leading to niche differentiation and promoting the co-existence of benthic fish species.

Key words: Bayesian analysis, food competition, niche differentiation, resource partitioning, stable isotopes

Introduction

In the last decade, online marketplaces have emerged as global distributors of alien species (Olden et al. 2021). The trade in ornamental fish is largely unregulated and both retailers as well as aquarists are often unaware of the risks of the release of invasive alien species into the wild (Padilla and Williams 2004). In addition, the network of interconnected waterways allows mobile and potentially invasive species to colonize new river catchments (Bij de Vaate et al. 2002; Van der Velde et al. 2002; Leuven et al. 2009). The most important pathways for the introduction of aquatic alien species into Western European freshwater ecosystems are the global ornamental pet trade (Van der Velde et al. 2002; Padilla and Williams 2004; Nunes et al. 2015; Chan et al. 2019; Olden et al. 2021) and connectivity of river catchments by the construction of canals that created invasion corridors between Eastern, Central, and Western Europe (Bij de Vaate et al. 2002; Van der Velde et al. 2002; Leuven et al. 2009).

Co-occurring species are compelled to share their habitat. Morphologically similar co-occurring fish species that live in sympatry often use niches that minimize competition (Giller 1984; McGill et al. 2006; Nagelkerke et al. 2018; Olivier et al. 2019). When an alien species enters an ecosystem, it can exploit an unoccupied niche and co-exist with native species through resource partitioning, where each species uses a different part of the shared resource or feeds at a different time or in a different location (Jackson and Britton 2014; Jackson et al. 2014). However, resource competition with native species may also occur, and in this case, the invader can rapidly dominate depending on the environmental conditions and behavioral adaptation (e.g., Van Riel et al. 2006; Van Kessel et al. 2016; Van der Loop 2022). This can lead to niche shifts, altering the food web and causing displacement of native species (Olden et al. 2004; Leuven et al. 2009; Rogosch and Olden 2020).

For risk assessment and risk prioritization of alien species, it is important to understand how alien species may impact native species and their food webs (Matthews 2017; Balzani and Haubrock 2022). Analyses of stable carbon and nitrogen isotopes of organisms and their food sources provide insight into their trophic levels and place in the food web and can be used to quantify isotopic niches (Newsome et al. 2007; Cucherousset et al. 2012; Jackson et al. 2012). The method can be employed to quantify energy sources and trophic structures of communities with which an indication of competition between sympatric species can be obtained.

In the present study, we focused on the trophic structure and dietary overlap of a Western European benthic fish community, *viz.* three native and two alien species in a lowland brook, using stable isotope analyses. One of the alien species is a weatherfish originating from East Asia. This is a popular species in the ornamental pet trade and has probably been introduced in the brook by (un)intentional release. Van Kessel et al. (2013) identified this first record of an alien weatherfish in the Netherlands as the oriental weatherfish (*Misgurnus anguillicaudatus*). A later genetic study demonstrated that it concerned the northern weatherfish (*M. bipartitus*) (Brys et al. 2020). This species belongs to a cryptic and unresolved taxonomic group of Cobitidae. The species has often been mistaken for *M. anguillicaudatus* due to its numerous morphological similarities (Belle et al. 2021; Clavero et al. 2023). The other alien species studied is the western tubenose goby (*Proterorhinus semilunaris*), originating from the Ponto-Caspian region (Cammaerts et al. 2012). This species has colonized the brook via upstream dispersal from the Meuse River.

Both alien fish species are still expanding their range in North-western Europe (Cammaerts et al. 2012; Manné et al. 2013; Binnendijk et al. 2017). A feeding morphology study demonstrated that the feeding capacities of several alien benthic species overlapped with those of native species, which makes competitive trophic interactions likely (Nagelkerke et al. 2018).

The present study aimed to determine the isotopic signature of the alien *M. bipartitus* and *P. semilunaris*, and the native stone loach (*Barbatula barbatula*), spined loach (*Cobitis taenia*), and gudgeon (*Gobio gobio*) and to identify whether the trophic niches of these benthic fish species overlap or indicate resource partitioning. Potential food competition among these species was further investigated by modeling their diet preferences. This provided an indication of competitive interactions between the native benthic fishes and the invaders.

Methods

Study site

The study site concerned a section of the lowland brook Tungalroyse beek (51°14.38'N, 005°52.086'E – 51°14.26'N, 005°47.77'E) near the village Tungalroy in the Province of Limburg, the Netherlands (Fig. 1). This brook of 35 km length has several tributaries and discharges into the Meuse River. The sampling site was selected because it harbors the largest known *M. bipartitus* population in the Netherlands (Binnendijk et al. 2017). Over most of its course, the brook was meandering and harbored well-developed riparian and hydrophyte vegetation. During sampling the mean water temperature was 16.0 °C, conductivity 664 µS/cm, pH 7.3, water velocity 0.2 m/s, depth 70 cm, and Secchi depth 60 cm. The brook width ranged between 5–8 m and its bed substrate predominantly consisted of sand.

Sampling

Samples of fish, macroinvertebrates, macrophytes, dead organic material, and bottom soil substrate were collected in October 2019 in the stretch of the brook that is denoted in Fig. 1. Fish were caught using handheld electrofishing equipment (Bretschneider EFGI 650). After catching, the fish were euthanized using a neutralized benzocaine solution of 100 mg l⁻¹ (Close et al. 1997; Neiffer and Stamper 2009; Department of Primary Industries 2015). Macroinvertebrates were collected using 70×55 cm dip nets with a mesh size of 1 mm. Helophytes, floating-leaved, and submerged aquatic macrophytes were collected by hand. Soil samples were collected by means of a hollow soil sampling tube with a diameter of 5 cm. Fish were identified using Kottelat and Freyhof (2007). Several keys were used for the identification of macroinvertebrates: amphipods (Zettler and Zettler 2017), crayfish (Koese and Soes 2011), dragonflies (Franke 1979), water beetles (Friday 1988), water bugs (Tempelman and Van Haaren 2009), and freshwater mollusks (Gittenberger et al. 2004). A Nikon SMZ800 stereo microscope with a 10–63 magnification was used for the identification of small macroinvertebrates. For the identification of macrophytes, we used the flora of Duistermaat (2020).

Sample preparation

All samples were transported to the laboratory and stored separately at -18 °C until preparation. To obtain muscle samples a piece of 0.5–1 cm of the dorsal tissue of each fish was dissected (Pinnegar and Polunin 1999). Muscle tissue samples were

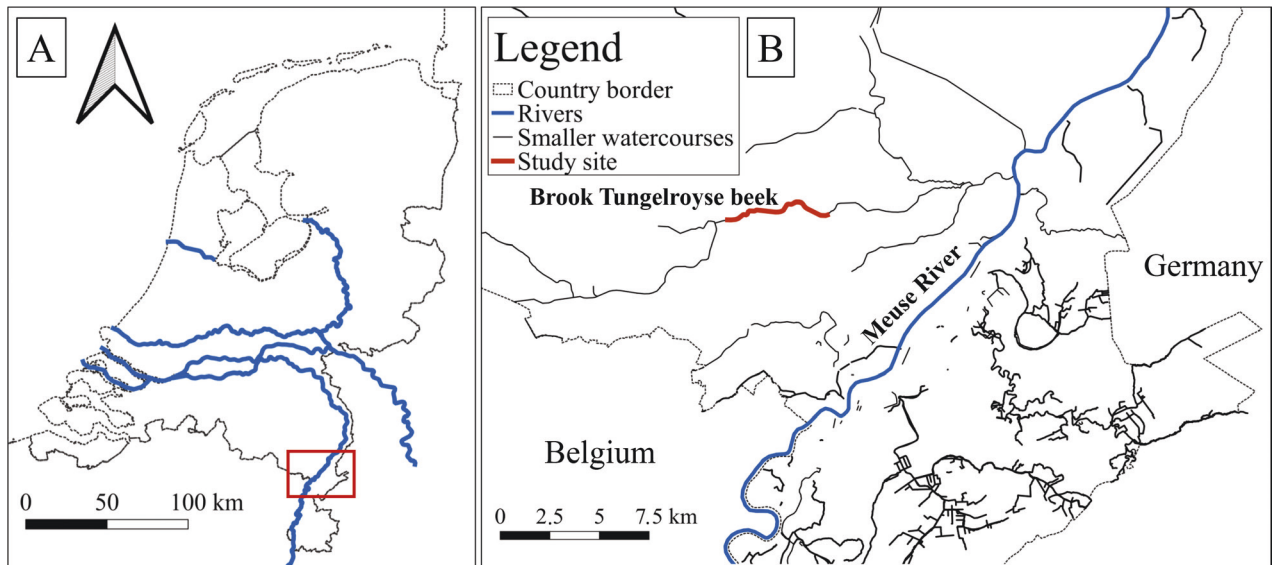


Figure 1. **A** The location of the study site in the Netherlands is framed in red. **B** Denotation of the sampled stretch of the brook Tungelroyse beek.

also dissected from crayfish and unionid mussels. For crayfish, the muscle tissue of the abdomen was used after the removal of the intestine. Other macroinvertebrates were stored alive for two days at 5 °C to empty their intestinal contents. Subsequently, these invertebrates were rinsed with tap water and after that with demineralized water before processing. From unionid mussels, muscle tissue of a similar size was dissected. Of small mollusks, whole soft bodies were used. For small mollusks and other macroinvertebrates, individuals of the same species were pooled to obtain enough material (0.22–0.26 mg) for analyses. The stems, leaves, and roots of individual plants were pooled.

After preparation, all samples were stored at -80 °C until freeze-drying. Freeze-drying was carried out at -90 °C for 24–48 hours for fish and macroinvertebrate samples (Paalvast and Van der Velde 2013; Verstijnen et al. 2019). Plant, dead organic material, and soil samples were freeze-dried for at least 48 hours. After freeze-drying, the samples were grounded with aluminum balls, for 2 min at 30 rpm, using a Retsch MM 400 (Verstijnen et al. 2019). Subsequently, the grounded samples were weighted in tin cups (Elemental Microanalysis 8 × 5 mm) and prepared for isotope analyses. For the fish and invertebrate samples, 0.22–0.26 mg dry weight was weighted. For plants and soil, separate samples were weighted for carbon (10 mg) and nitrogen (40 mg) analyses.

Stable isotope analyses

Carbon and nitrogen stable isotopes were measured using a Thermo Scientific FLASH 2000 HT Elemental Analyzer with a Thermo Scientific DELTA V Advantage Next Generation Isotope Ratio mass spectrometer. Reference gasses were calibrated with the IAEA standards (IAEA-N-2 and IAEA-CH-6), with a maximum deviation of 0.15‰. As an internal standard control, caffeine was used and the $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ of every sample were determined (in ‰). The isotope ratios (R) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are relative to Vienna PDB and atmospheric N_2 and were calculated by:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) * 1000$$

Analyses of trophic levels

Quantification of energy sources and trophic structure of communities and organisms was conducted by comparing relationships of naturally occurring stable isotope ratio distributions of nitrogen $^{15}\text{N}:^{14}\text{N}$ and carbon $^{13}\text{C}:^{12}\text{C}$. Stable isotope ratios of nitrogen ($\delta^{15}\text{N}$) are enriched between prey and predator tissues, thus providing a measure for the trophic position of a consumer in a food web. The trophic position of each fish species in the food web was calculated following Vander Zanden and Rasmussen (1999). Stable isotope ratios of carbon ($\delta^{13}\text{C}$) show little or no trophic level enrichment and are useful for identifying the production sources for consumers (Vander Zanden et al. 1999). For calculating the primary consumer $\delta^{15}\text{N}-\delta^{13}\text{C}$ baseline, the $\delta^{15}\text{N}$ (‰) measured values of the mollusks were used (Table 1).

Statistical analyses

Convex hull areas were used to display isotopic overlap between fish, mollusks, other macroinvertebrates, and plants. Isotopic niche widths of benthic species were compared using Bayesian standard ellipse areas (SEA) (Jackson et al. 2011; Syväranta et al. 2013). SEA are less sensitive to increasing sample sizes, whereas Convex hull areas tend to increase as the sample size increases for comparing isotopic niche widths. SEA with a 95% confidence interval of benthic fish species and overlap between SEA were computed using the Stable Isotope Bayesian Ellipses in R (SIBER) package (Jackson et al. 2011). This approach calculates Bayesian SEA parameters by running 100,000 Markov-Chain Monte Carlo (MCMC) simulations. Subsequently, isotopic niche areas (‰²) and proportion overlap (%) were estimated on the posterior distribution of the covariance matrix for each benthic fish species and compared. The estimates were corrected for sample size (SEAc) by two standard deviations and plotted (Jackson et al. 2011; Ryan et al. 2013). The relationships between benthic fish length and their $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signatures (‰) were analyzed using Pearson correlation tests.

Modeling benthic fish species diet source proportion was done with a Bayesian mixing model that generates robust probability estimates using the Stable Isotope Analysis in R (SIAR) package (Parnell et al. 2010; Parnell and Jackson 2013). The initial diet sources concerned all sampled macroinvertebrates with the exemption of spiny-cheeked crayfish (*Faxonius limosus*) > 40 mm since they are too large to be a prey of the benthic fish species of concern. Thus, the model was first run with a wide range of 28 possible diet sources per consumer. Via a backward stepwise selection procedure, a dietary item that contributed the least was then excluded. Eventually, stable isotope signatures of the five most important dietary items were included in a final model. It is never clear what the accurate fractional contribution of an isotopic source is to a consumer's tissue (Martínez del Rio et al. 2009). Therefore, we modeled proportions of diet sources for the consumers on the basis of four different stable isotope fractionation factors (ΔN and ΔC) and associated standard deviations, in accordance with Verstijnen et al. (2019). These are: A) ΔN 3.4 ± 1.0 and ΔC 0.4 ± 1.3 (Post 2002: established mean trophic fractionation based on a literature review), B) ΔN 2.9 ± 1.2 and ΔC 1.3 ± 1.3 (McCutchan et al. 2003: fractionation in muscle tissue), C) ΔN 2.3 ± 1.6 and ΔC 0.4 ± 1.2 (McCutchan et al. 2003: aquatic consumers) and D) ΔN 3.1 ± 1.6 and ΔC 2.0 ± 0.8 (Caut et al. 2009: based on a selection of data of fractionation in muscle tissue of fish). Computing all analyses and statistical tests was done with the program R version 3.6.2 (R Core Team 2021).

Table 1. Sample size, length range (mm), mean isotopic of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (‰) signature, standard deviation and range per species or taxon, alphabetically ordered. Alien species are indicated in bold, benthic fish species are depicted with a *. The fish length was measured from the tip of the snout to the tip of the tail (TL). Sampling location: Tungelroyse beek.

Specimen	n	Length range (mm)	Arithmetic mean		Standard deviation		Isotopic range	
			$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$
Fish								
<i>Barbatula barbatula</i> *	14	48–91	16.33	-32.41	2.27	1.32	11.82–19.06	-33.90 – -28.79
<i>Cobitis taenia</i> *	19	48–106	16.38	-32.54	1.77	0.98	13.26–19.34	-34.18 – -31.25
Cyprinidae juv.	10	27–34	18.29	-32.52	0.54	1.05	17.54–18.97	-34.16 – -31.05
<i>Cyprinus carpio</i>	5	62–83	17.70	-30.48	0.65	0.71	16.97–18.66	-31.42 – -29.76
<i>Esox lucius</i>	4	240–462	18.09	-32.81	0.70	0.49	17.06–18.60	-33.23 – -32.13
<i>Gasterosteus aculeatus</i>	27	33–44	18.64	-31.11	1.21	1.17	16.54–20.15	-34.99 – -29.67
<i>Gobio gobio</i> *	15	32–125	17.14	-31.36	2.62	0.96	11.07–19.78	-33.63 – -29.68
<i>Lepomis gibbosus</i>	2	21–78	15.96	-33.43	2.95	0.27	13.88–18.05	-33.62 – -33.24
<i>Misgurnus bipartitus</i>*	25	39–162	16.97	-30.97	1.31	0.73	13.63–18.76	-33.03 – -29.55
<i>Perca fluviatilis</i>	7	67–94	17.97	-33.12	1.38	1.18	15.71–19.44	-34.69 – -30.94
<i>Proterorhinus semilunaris</i>*	34	29–68	17.30	-32.58	0.95	1.31	14.52–18.54	-36.12 – -30.56
<i>Pseudorasbora parva</i>	4	52–69	14.26	-28.70	3.75	2.05	8.63–16.27	-29.90 – -25.64
<i>Pungitius pungitius</i>	1	34	15.39	-31.06	n.a.	n.a.	15.39–15.39	-31.06 – -31.06
<i>Rhodeus amarus</i>	24	34–65	16.75	-35.67	2.23	1.74	12.60–19.16	-39.29 – -33.44
<i>Rutilus rutilus</i>	25	43–172	16.82	-33.74	2.06	1.67	12.90–20.27	-36.13 – -30.89
<i>Silurus glanis</i>	6	89–357	19.23	-32.54	0.48	0.61	18.55–19.83	-33.52 – -31.94
<i>Tinca tinca</i>	1	181	17.45	-31.74	n.a.	n.a.	17.45–17.45	-31.74 – -31.74
<i>Umbra pygmaea</i>	1	62	13.82	-32.17	n.a.	n.a.	13.82–13.82	-32.17 – -32.17
Macroinvertebrates								
<i>Aeshna mixta</i> larvae	1	n.a.	8.03	-33.67	n.a.	n.a.	8.03–8.03	-33.67 – -33.67
<i>Anax</i> sp. larvae	6	n.a.	18.34	-32.50	0.51	0.53	17.76–18.86	-33.28 – -32.01
Anisoptera larvae	1	n.a.	15.29	-30.76	n.a.	n.a.	15.29–15.29	-30.76 – -30.76
<i>Bithynia tentaculata</i>	1	n.a.	14.02	-32.00	n.a.	n.a.	14.02–14.02	-32.00 – -32.00
<i>Calopteryx splendens</i> larvae	11	n.a.	17.21	-33.26	0.58	1.04	16.24–18.28	-34.78 – -31.79
Chironomid larvae	1	n.a.	13.47	-32.76	n.a.	n.a.	13.47–13.47	-32.76 – -32.76
<i>Corbicula leana</i>	11	11–23	14.59	-35.17	0.50	1.16	13.76–15.24	-37.04 – -33.56
<i>Cybister lateralmarginalis</i> larvae	1	n.a.	16.34	-35.12	n.a.	n.a.	16.34–16.34	-35.12 – -35.12
<i>Faxonius limosus</i> <40 mm	9	32–39	17.05	-31.68	0.38	0.62	16.19–17.43	-32.53 – -30.39
<i>Faxonius limosus</i> 40–60 mm	19	40–60	16.61	-31.38	0.88	1.04	14.38–17.53	-32.96 – -29.18
<i>Faxonius limosus</i> >60 mm	8	61–84	16.20	-32.34	1.52	1.58	13.50–18.18	-34.93 – -30.00
<i>Gammarus roeselii</i>	1	n.a.	14.35	-30.78	n.a.	n.a.	14.35–14.35	-30.78 – -30.78
<i>Ilyocoris cimicoides</i>	3	n.a.	16.94	-31.34	0.47	0.26	16.40–17.24	-31.49 – -31.04
<i>Ischnura elegans</i>	1	n.a.	17.88	-32.58	n.a.	n.a.	17.88–17.88	-32.58 – -32.58
<i>Lymnaea stagnalis</i>	6	n.a.	11.31	-33.45	2.33	1.55	8.69–14.56	-35.28 – -30.90
<i>Micronecta scholtzi</i>	1	n.a.	15.32	-33.75	n.a.	n.a.	15.32–15.32	-33.75 – -33.75
<i>Nepa cinerea</i>	6	n.a.	15.29	-30.07	0.89	1.62	14.28–16.53	-32.70 – -27.81
<i>Notonecta glauca</i>	14	n.a.	13.85	-29.68	2.57	1.83	8.16–16.29	-32.93 – -26.35
<i>Notonecta viridis</i>	3	n.a.	7.09	-28.63	2.41	2.41	4.33–8.76	-30.08 – -25.85
<i>Physa acuta</i>	6	n.a.	16.21	-29.43	0.72	2.02	15.31–17.13	-32.31 – -26.08
<i>Planorbarius corneus</i>	2	n.a.	13.73	-30.97	1.06	1.32	12.98–14.48	-31.90 – -30.03

Specimen	n	Length range (mm)	Arithmetic mean		Standard deviation		Isotopic range	
			$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$
<i>Potamopyrgus antipodarum</i>	1	n.a.	15.82	-21.13	n.a.	n.a.	15.82–15.82	-21.13 – -21.13
<i>Radix auricularia</i>	5	n.a.	15.52	-35.32	0.97	1.29	14.41–17.05	-37.10 – -33.65
<i>Radix labiata</i>	2	n.a.	13.26	-34.15	3.61	0.26	10.71–15.81	-34.34 – -33.97
<i>Ranatra linearis</i>	1	n.a.	12.92	-30.86	n.a.	n.a.	12.92–12.92	-30.86 – -30.86
<i>Rhantus suturalis</i>	2	n.a.	13.19	-29.75	3.11	4.80	10.99–15.39	-33.15 – -26.36
<i>Sialis lutaria</i>	1	n.a.	13.60	-32.41	n.a.	n.a.	13.60–13.60	-32.41 – -32.41
<i>Sigara falleni</i>	2	n.a.	15.24	-33.92	0.32	0.32	15.01–15.47	-34.15 – -33.69
<i>Sphaerium</i> sp.	2	n.a.	14.26	-29.32	0.71	0.15	13.76–14.76	-29.43 – -29.22
<i>Unio pictorum</i>	4	52–75	14.08	-35.35	0.44	0.62	13.44–14.43	-36.18 – -34.75
Macrophytes								
<i>Alisma plantago-aquatica</i>	2	n.a.	7.53	-28.91	1.60	0.44	6.40–8.67	-29.22 – -28.60
<i>Azolla filiculoides</i>	1	n.a.	10.65	-31.64	n.a.	n.a.	10.65–10.65	-31.64 – -31.64
<i>Bidens cernua</i>	1	n.a.	14.36	-32.95	n.a.	n.a.	14.36–14.36	-32.95 – -32.95
<i>Callitriche</i> sp.	2	n.a.	14.92	-33.50	1.34	2.10	13.97–15.86	-34.98 – -32.01
<i>Ceratophyllum demersum</i>	2	n.a.	14.88	-35.29	0.24	4.89	14.71–15.06	-38.75 – -31.83
Degraded plant material	4	n.a.	15.99	-32.04	0.87	0.79	15.01–17.12	-32.61 – -30.93
<i>Elodea nuttallii</i>	1	n.a.	12.78	-31.94	n.a.	n.a.	12.78–12.78	-31.94 – -31.94
<i>Equisetum palustre</i>	2	n.a.	12.00	-29.95	1.05	0.43	11.26–12.74	-30.26 – -29.65
<i>Glyceria fluitans</i>	2	n.a.	16.42	-32.50	0.33	0.07	16.18–16.65	-32.55 – -32.45
<i>Glyceria maxima</i>	2	n.a.	10.97	-30.83	0.22	0.70	10.81–11.12	-31.32 – -30.33
<i>Hydrocharis morsus-ranae</i>	1	n.a.	17.26	-30.20	n.a.	n.a.	17.26–17.26	-30.20 – -30.20
<i>Juncus effusus</i>	1	n.a.	15.08	-31.17	n.a.	n.a.	15.08–15.08	-31.17 – -31.17
<i>Mentha aquatica</i>	3	n.a.	15.90	-32.76	1.01	0.08	14.75–16.62	-32.84 – -32.69
<i>Persicaria hydropiper</i>	2	n.a.	12.43	-32.05	0.19	0.20	12.30–12.57	-32.19 – -31.91
<i>Persicaria</i> sp.	1	n.a.	17.00	-32.21	n.a.	n.a.	17.00–17.00	-32.21 – -32.21
<i>Phalaris arundinacea</i>	3	n.a.	9.49	-29.66	2.60	0.84	7.73–12.48	-30.41 – -28.75
Plant material indet.	6	n.a.	15.25	-33.96	2.34	3.37	12.07–18.77	-39.95 – -31.29
<i>Potamogeton natans</i>	2	n.a.	10.49	-31.70	1.61	0.41	9.35–11.63	-31.99 – -31.41
<i>Sparganium erectum</i>	1	n.a.	13.78	-30.83	n.a.	n.a.	13.78–13.78	-30.83 – -30.83
<i>Tanacetum vulgare</i>	1	n.a.	17.16	-32.92	n.a.	n.a.	17.16–17.16	-32.92 – -32.92
<i>Typha latifolia</i>	1	n.a.	7.28	-30.68	n.a.	n.a.	7.28–7.28	-30.68 – -30.68
Algae								
Chlorophyta filamentous algae	2	n.a.	15.11	-34.81	3.23	0.68	12.82–17.39	-35.29 – -34.33
Soil								
Soil	20	n.a.	6.31	-26.83	2.42	4.70	-0.49–9.68	-30.55 – -13.55

Results

In total, 18 fish species including five benthic ones, 28 macroinvertebrate taxa, 20 plant taxa (including decaying organic plant material), one taxon of filamentous green algae and 20 soil samples were collected and analyzed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope ratios (Table 1). The food web shows distinct $\delta^{13}\text{C}$ signatures (Fig. 2). Four trophic levels can be distinguished. $\delta^{15}\text{N}$ enrichment was encountered the least in the soil samples. Gradually, soil, macrophytes (including dead organic plant material), algae, macroinvertebrates, and fish appeared to be more $\delta^{15}\text{N}$ enriched.

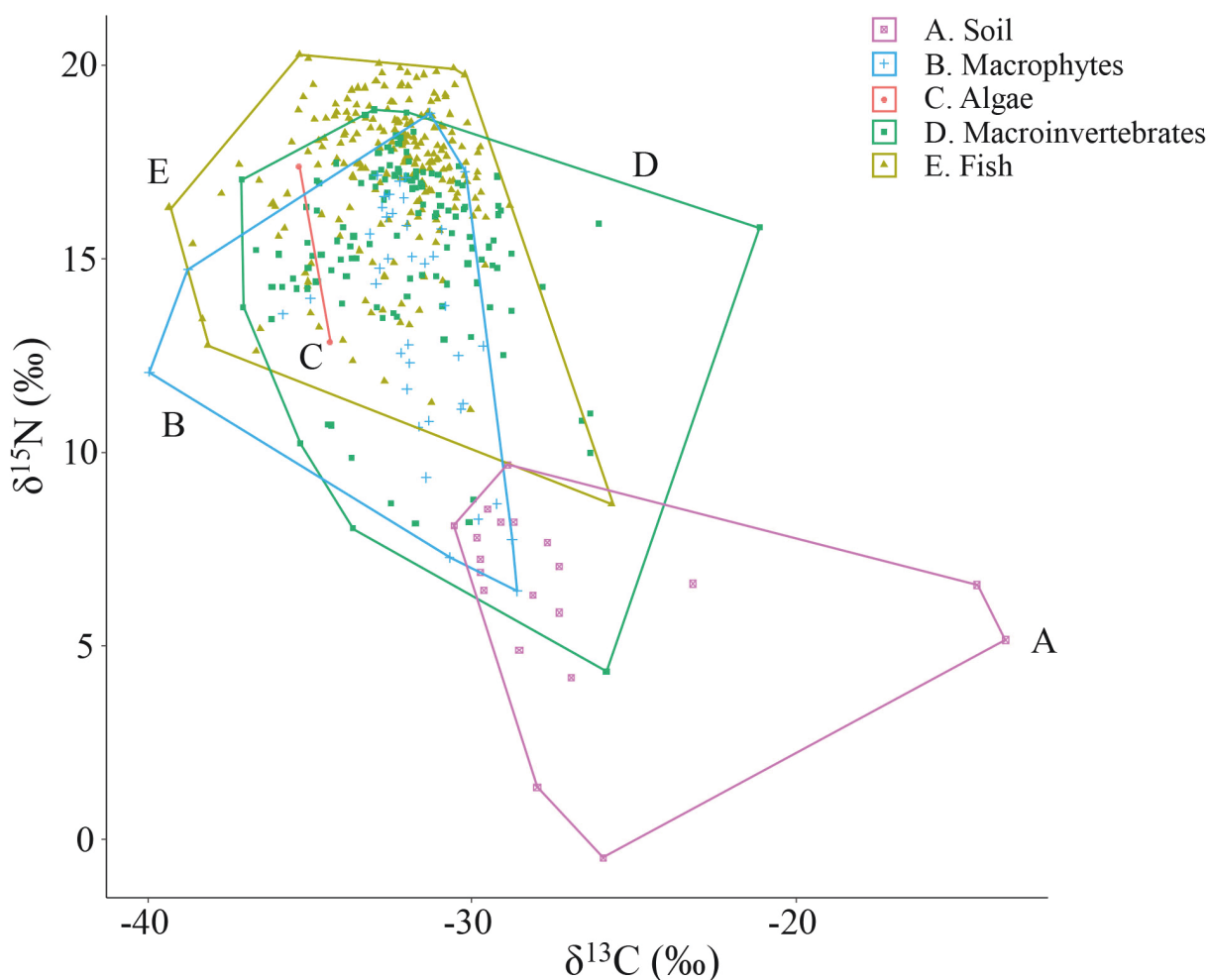


Figure 2. Figure of the food web of the brook Tungelroyse beek depicting $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (‰) isotopic signatures of all sampled organisms and soil samples. The number of used individuals per species or taxa, and associated standard deviations and ranges are presented in Table 1.

Trophic positions of fish

In total, eight fish species were particularly abundant: *Barbatula barbatula*, *Cobitis taenia*, *Gobio gobio*, *Gasterosteus aculeatus*, *M. bipartitus*, *P. semilunaris*, *Rhodeus amarus*, and *Rutilus rutilus* (Table 1). *Barbatula barbatula*, *C. taenia*, *G. gobio*, and *P. semilunaris* were caught at the bottom of the brook, mostly on bare (unvegetated) bed substrate. *Rhodeus amarus* and *R. rutilus* were mostly caught in vegetated zones of the brook. *Misgurnus bipartitus* was most frequently caught in dense vegetation zones close to the banks. *Gasterosteus aculeatus* was caught in several habitats. The isotopic signatures of all sampled fish ranged between -39.29 (*R. amarus*) and -25.64 (*P. parva*) for $\delta^{13}\text{C}$, for $\delta^{15}\text{N}$ this ranged between 8.63 (*P. parva*) and 20.27 (*R. rutilus*).

The $\delta^{15}\text{N}$ signature of fish demonstrates that *Silurus glanis* is most likely the top predator in the brook Tungelroyse beek (Fig. 3). It also showed the highest mean trophic position of the fish community (Fig. 4). Individual trophic positions varied between 1.24 (*G. gobio* individual) up to 3.90 (*G. aculeatus* individual). Of the 14 fish species included in the cluster analysis, *M. bipartitus* ranked 8th with a mean trophic position of 2.9 , respectively preceded and followed by the benthic *G. gobio* (3.0) and *P. semilunaris* (2.9). The two other benthic species *B. barbatula*

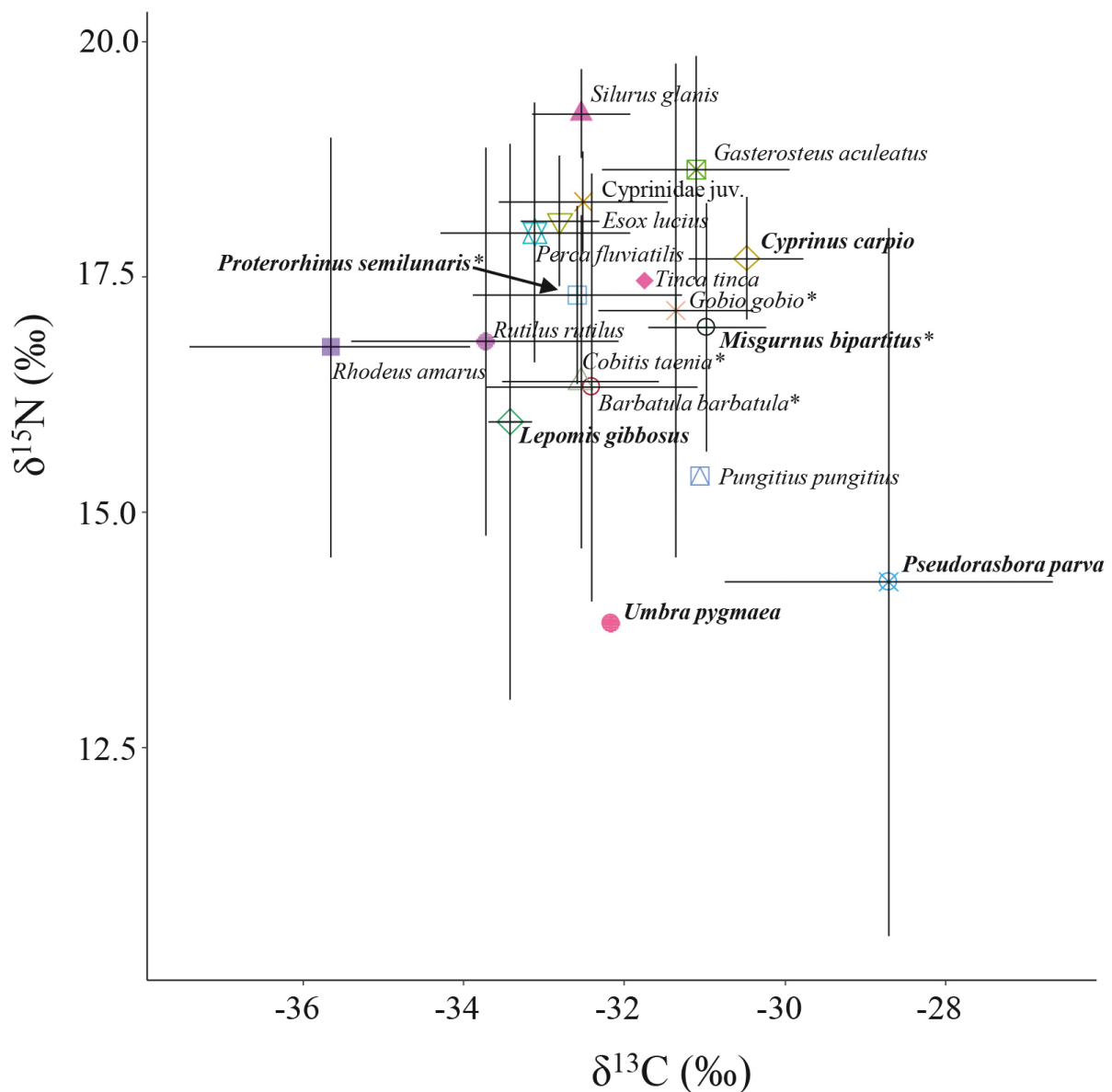


Figure 3. Figure of trophic signatures of fish species and taxa depicting mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values (‰). The lines denote the standard deviations. The number of used individuals per species or taxa, and associated standard deviations and ranges are presented in Table 1. Benthic fish species are depicted with a * and alien species are indicated in bold.

and *C. taenia* showed a lower mean trophic level of 2.6. Cluster analysis based on mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values shows that four groups can be distinguished and two species occupy a distinct position (Fig. 5). The latter two species concern a relatively small planktivorous species (*P. parva*) and a small predominantly herbivorous cyprinid (*R. amarus*) that forages mainly on diatoms. Group 1 consists of an omnivore (*R. rutilus*) and three benthivores (*B. barbatula*, *C. taenia*, *P. semilunaris*). Group 2 consists of two benthivorous species (*G. gobio*, *M. bipartitus*) and an omnivore (*Cyprinus carpio*). Group 3 consists of two piscivorous species (*Esox lucius*, *Perca fluviatilis*) and zooplankton feeding juvenile cyprinids that were too small to identify at the species level. Group 4 consists of two insectivorous and piscivorous species (*G. aculeatus*, *S. glanis*).

Pearson correlation analyses of all three native benthic fish species revealed that body size correlated negatively in a significant way with their $\delta^{15}\text{N}$ signatures: *B. barbatula*

($r = -0.60$, $t(12) = -2.56$, $p < 0.05$), *C. taenia* ($r = -0.54$, $t(17) = -2.67$, $p < 0.05$), and *G. gobio* ($r = -0.70$, $t(13) = -3.56$, $p < 0.01$) (Fig. 6). In addition, significantly positive correlations between body size and $\delta^{13}\text{C}$ signatures were observed for *C. taenia* ($r = 0.58$, $t(17) = 2.92$, $p < 0.01$) and *G. gobio* ($r = 0.68$, $t(13) = 3.32$, $p < 0.01$).

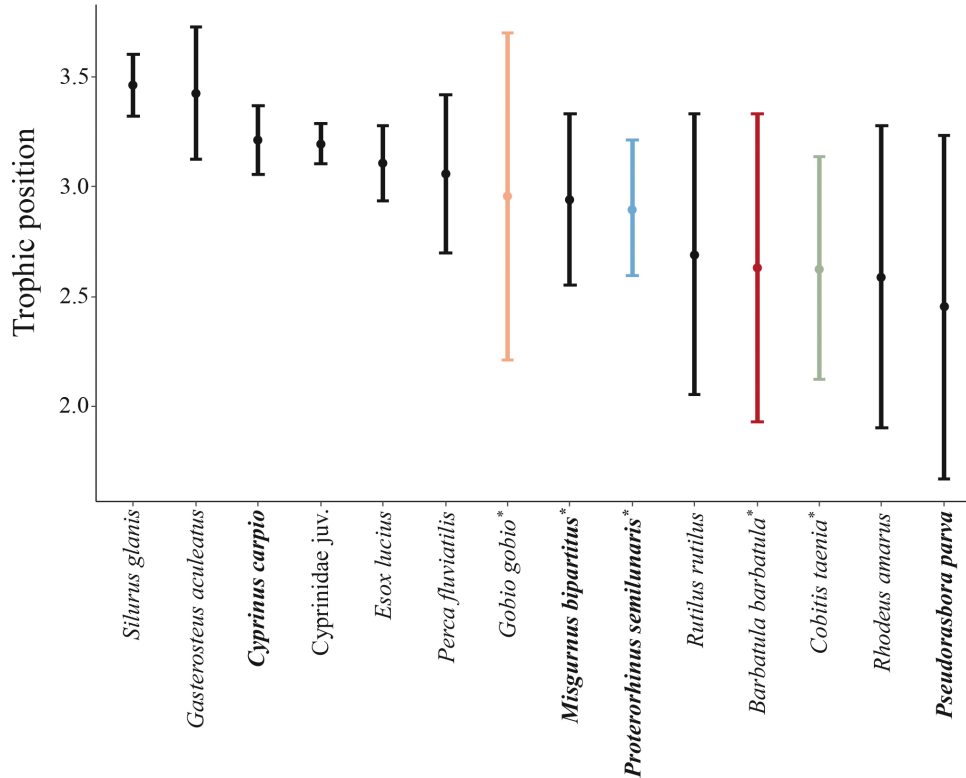


Figure 4. Figure of the mean trophic position of fish species and taxa for which 4 or more individuals were collected. Bars represent standard deviations. Benthic fish species are depicted with a * and alien species are indicated in bold.

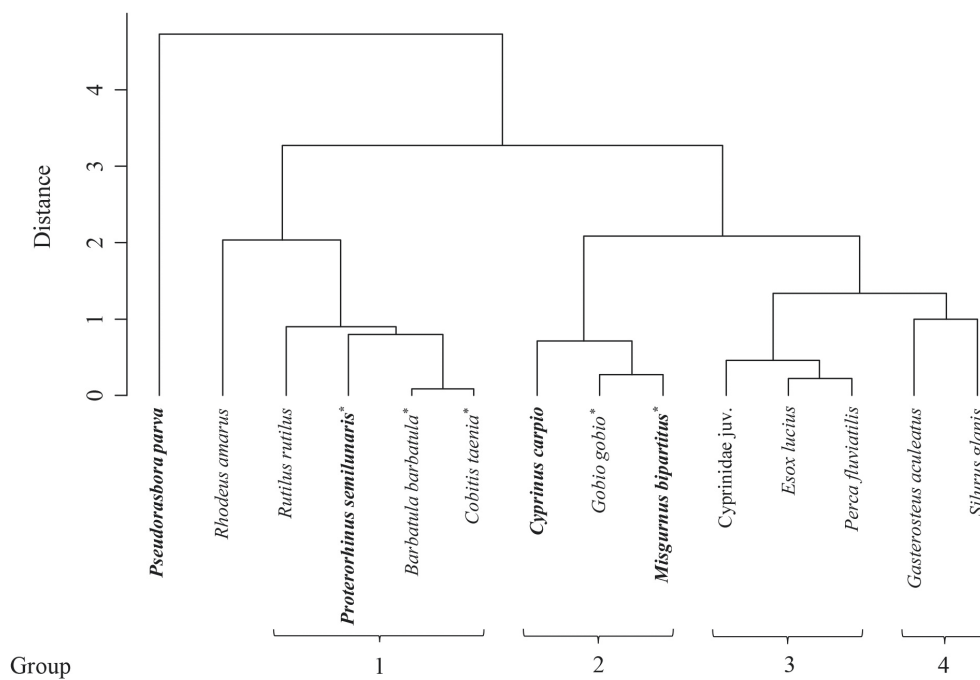


Figure 5. Dendrogram obtained from complete linkage cluster analysis (Euclidean distance) of mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for fish species or taxa of which 4 or more individuals were encountered. Benthic fish species are depicted with a * and alien species are indicated in bold.

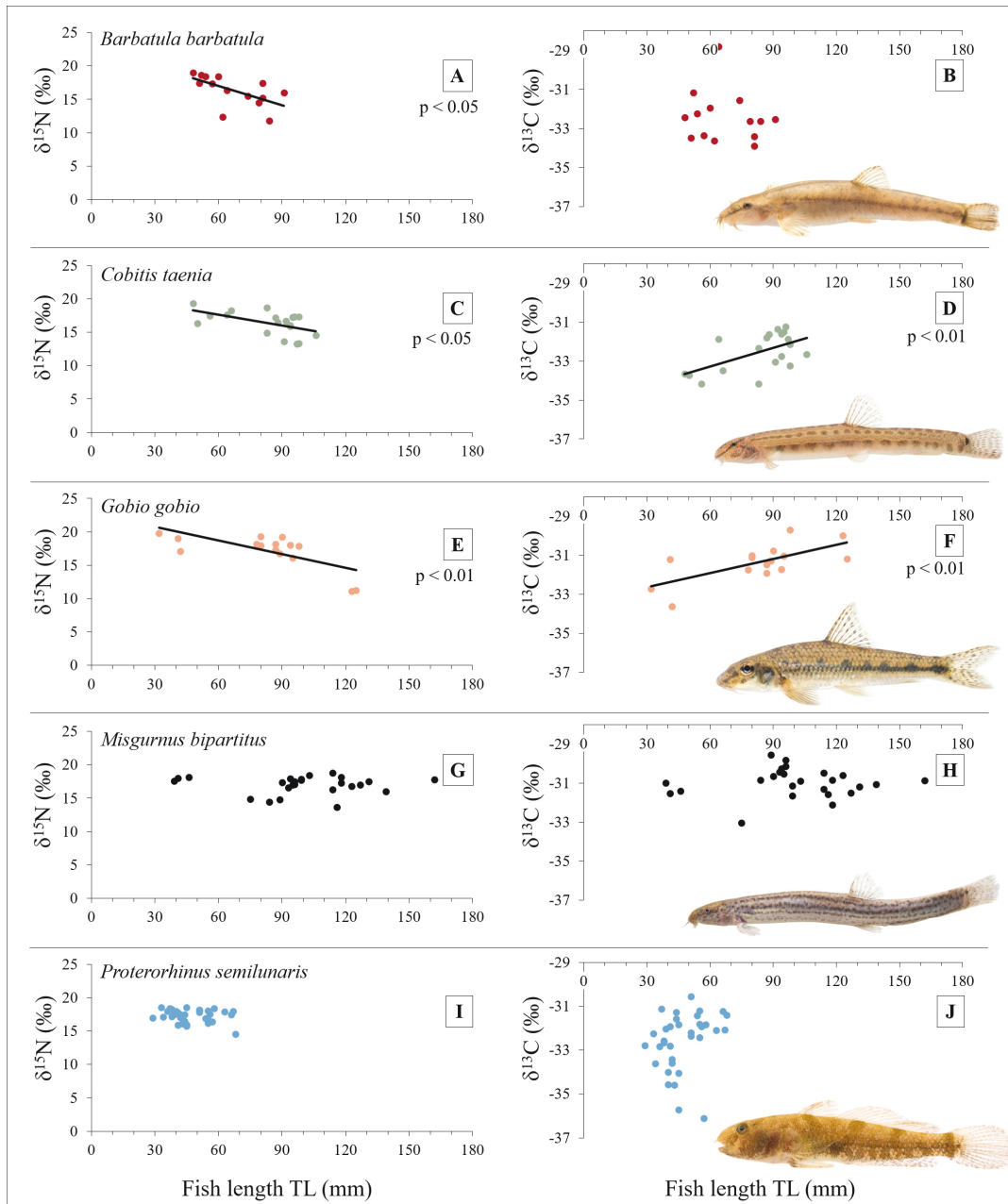


Figure 6. Correlations between total length (mm) and stable isotope ratios ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in ‰) of five benthic fish species in the brook Túngelroyse beek. Significant Pearson correlations are indicated with a p-value and a trend line.

Benthic fish niche space and area

The niche space of five benthic species: *B. barbatula*, *C. taenia*, *G. gobio*, *M. bipartitus*, and *P. semilunaris* was determined using the 95% confidence interval Bayesian standard ellipses of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (‰) (Fig. 7). Juvenile and adult individuals were included in this analysis. Isotopic niche area overlap was observed among all benthic species. The largest isotopic niche area was observed for *B. barbatula* and the smallest for *P. semilunaris* and *M. bipartitus*, respectively (Fig. 8). *Misgurnus bipartitus* displayed a significant niche overlap with *G. gobio* (91.8%), *B. barbatula* (37.1%), *P. semilunaris* (8.9%), and *C. taenia* (1.1%) (Table 2). *Proterorhinus semilunaris* displayed significant isotopic overlap with *B. barbatula* (91.2%), *C. taenia* (66.1%), *G. gobio* (37.4%), and *M. bipartitus* (8.9%).

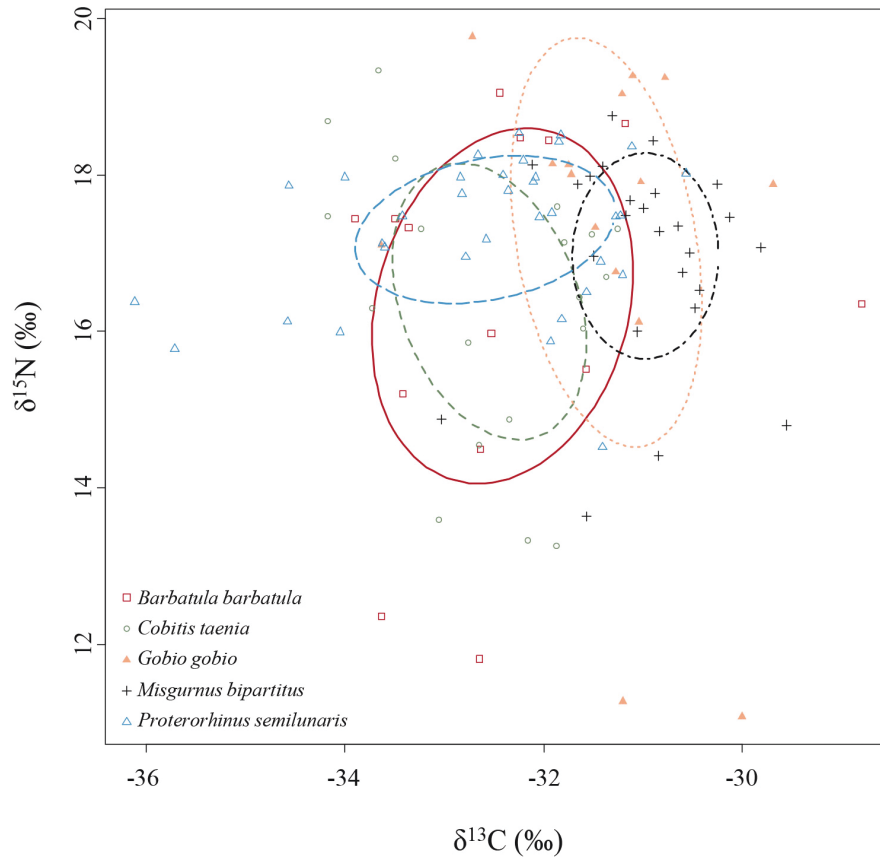


Figure 7. The 95% confidence interval corrected Bayesian standard ellipse areas (SEAc) of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (‰), demonstrating significant isotopic niche partitioning among five benthic fish species in the brook Túngelroyse beek.

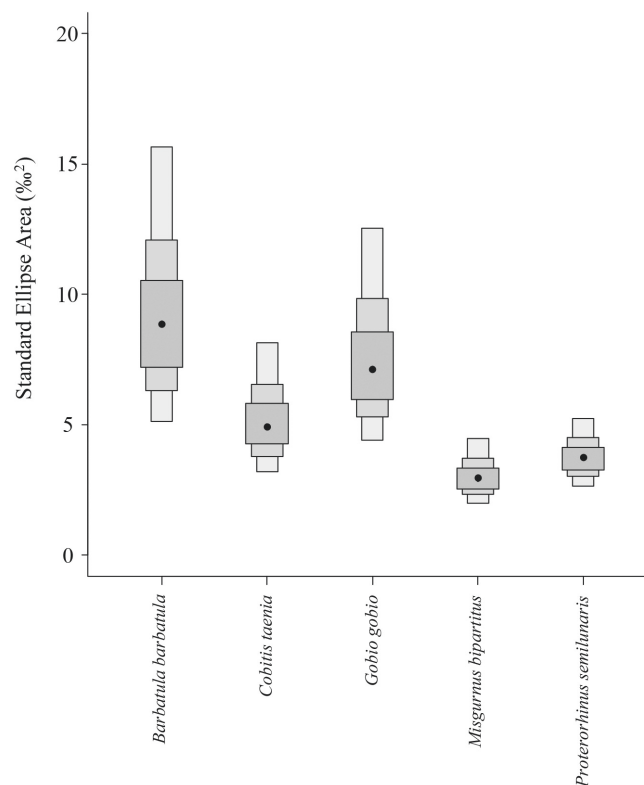


Figure 8. Measures of uncertainty and central tendency of corrected Bayesian standard ellipse areas ($\%^{2}$) based on 100,000 posterior draws of parameters showing 95, 75, and 50% credibility intervals from light to dark grey respectively. Black dots denote the mean.

Table 2. Isotopic niche area (‰²) and proportion overlap (%) of corrected Bayesian standard ellipse areas (SEAc) between five benthic fish species in the brook Tungelroyse beek. n represents the number of individuals per species used in the analyses.

Species	Isotopic niche area SEAc (‰ ²)	Proportion SEAc overlap (%)				
		<i>Barbatula barbatula</i>	<i>Cobitis taenia</i>	<i>Gobio gobio</i>	<i>Misgurnus bipartitus</i>	<i>Proterorhinus semilunaris</i>
<i>Barbatula barbatula</i> (n = 14)	9.99					
<i>Cobitis taenia</i> (n = 19)	5.47	99.2				
<i>Gobio gobio</i> (n = 15)	8.11	44.6	15.6			
<i>Misgurnus bipartitus</i> (n = 25)	3.16	37.1	1.1	91.8		
<i>Proterorhinus semilunaris</i> (n = 34)	3.89	91.2	66.1	37.4	8.9	

Modelling benthic fish species diet

Using Bayesian mixing models, the diet source proportion of the five benthic fish species with four different fractionation factors was modeled. The estimation of five out of 28 possible dietary items that mostly contributed to the diet elucidated notable differences in the diet of alien and native fish species (Table 3). Between different fractionation factors, the model generally produced a similar output. For *B. barbatula*, the most important dietary items were the snails *Lymnaea stagnalis* and *Bithynia tentaculata*, the water boatman *Sigara falleni*, larvae of non-biting midges (Chironomidae), and the small water boatman *Micronecta scholtzi* made up 18–30%, 11–24%, 14–23%, 16–22%, and 14–21% of the diet, respectively. The most important dietary items of *C. taenia* were *B. tentaculata* (6–34%), *L. stagnalis* (16–31%), the snail *Radix labiata* (13–27%), *S. falleni* (11–21%), and *M. scholtzi* (11–19%). For *G. gobio*, these were chironomid larvae (15–34%), larvae of the damselfly *Calopteryx splendens* (14–31%), the snail *Planorbarius corneus* (9–23%), the water stick insect *Ranatra linearis* (9–23%), and *B. tentaculata* (17–21%). The most important dietary items of *M. bipartitus* were chironomid larvae (15–75%), dragonfly (Anisoptera) larvae (4–32%), *B. tentaculata* (13–29%), *R. linearis* (3–29%), and *Gammarus roeselii* (4–21%). Due to the small size of their mouth, *B. barbatula*, *C. taenia*, and *M. bipartitus* probably only eat small individuals of snails. For *P. semilunaris*, the most important dietary items were *Corbicula leana* (15–50%), *S. falleni* (11–40%), *B. tentaculata* (10–27%), *G. roeselii* (5–25%), and *R. labiata* (5–22%).

The mixing models showed that the native species *B. barbatula* and *C. taenia* had a similar diet since they had four out of five most important dietary items in common (Table 3). These were two snails and two boatman species. The native *G. gobio* had three dietary items in common with the alien *M. bipartitus*. Chironomid larvae were the most important dietary item for both species. The alien *P. semilunaris* had three dietary items in common with *C. taenia*: two snails and a boatman. The dietary items that appear to be the most important for *C. taenia* were less important for *P. semilunaris* (Table 3).

Discussion

Isotopic niche and food overlap of native and alien species

Analyses of stable isotope ratios ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) have provided insight into the food web of a Western European lowland brook. Two alien benthic fish species have been established in the brook Tungelroyse beek in the last two decades (NDFP

Table 3. Diet proportion of five benthic fish species dietary item (lower 95% interval-higher 95% interval) in the brook Túngelroyse beek. The five most important dietary items were determined using Bayesian mixing models. The presented proportions are based on four different fractionation factors* (A, B, C, and D), where the highest determined proportion per dietary item is depicted in bold.

Dietary item	Mean proportion per fractionation factor*			
	A	B	C	D
Consumer: <i>Barbatula barbatula</i>				
<i>Bithynia tentaculata</i>	0.23 (0.01–0.43)	0.13 (0.00–0.30)	0.24 (0.02–0.44)	0.11 (0.00–0.28)
Chironomid larvae	0.22 (0.00–0.42)	0.18 (0.00–0.37)	0.21 (0.00–0.41)	0.16 (0.00–0.35)
<i>Lymnaea stagnalis</i>	0.27 (0.03–0.49)	0.27 (0.04–0.48)	0.18 (0.00–0.35)	0.30 (0.06–0.53)
<i>Micronecta scholtzi</i>	0.14 (0.00–0.32)	0.20 (0.00–0.40)	0.19 (0.00–0.36)	0.21 (0.00–0.40)
<i>Sigara falleni</i>	0.14 (0.00–0.31)	0.21 (0.00–0.40)	0.18 (0.00–0.35)	0.23 (0.00–0.43)
Consumer: <i>Cobitis taenia</i>				
<i>Bithynia tentaculata</i>	0.31 (0.11–0.51)	0.10 (0.00–0.24)	0.34 (0.14–0.55)	0.06 (0.00–0.17)
<i>Lymnaea stagnalis</i>	0.31 (0.11–0.51)	0.25 (0.05–0.42)	0.16 (0.00–0.32)	0.26 (0.04–0.45)
<i>Micronecta scholtzi</i>	0.11 (0.00–0.28)	0.18 (0.00–0.37)	0.19 (0.00–0.37)	0.17 (0.00–0.36)
<i>Radix labiata</i>	0.16 (0.00–0.36)	0.27 (0.03–0.47)	0.15 (0.00–0.34)	0.31 (0.05–0.53)
<i>Sigara falleni</i>	0.11 (0.00–0.27)	0.20 (0.00–0.38)	0.17 (0.00–0.36)	0.21 (0.00–0.39)
Consumer: <i>Gobio gobio</i>				
<i>Bithynia tentaculata</i>	0.21 (0.00–0.41)	0.20 (0.00–0.40)	0.19 (0.00–0.39)	0.17 (0.00–0.38)
Odonata (<i>Calopteryx splendens</i>) larvae	0.14 (0.00–0.29)	0.28 (0.07–0.48)	0.21 (0.02–0.38)	0.31 (0.06–0.56)
Chironomid larvae	0.19 (0.00–0.38)	0.28 (0.02–0.52)	0.15 (0.00–0.33)	0.34 (0.05–0.62)
<i>Planorbarius corneus</i>	0.23 (0.00–0.41)	0.12 (0.00–0.30)	0.23 (0.01–0.42)	0.09 (0.00–0.25)
<i>Ranatra linearis</i>	0.23 (0.00–0.42)	0.12 (0.00–0.28)	0.21 (0.00–0.39)	0.09 (0.00–0.25)
Consumer: <i>Misgurnus bipartitus</i>				
<i>Bithynia tentaculata</i>	0.19 (0.00–0.37)	0.29 (0.02–0.53)	0.21 (0.00–0.41)	0.13 (0.00–0.35)
Chironomid larvae	0.19 (0.02–0.35)	0.45 (0.25–0.65)	0.15 (0.00–0.31)	0.75 (0.52–0.95)
<i>Gammarus roeselii</i>	0.20 (0.00–0.37)	0.09 (0.00–0.23)	0.21 (0.01–0.41)	0.04 (0.00–0.11)

Dietary item	Mean proportion per fractionation factor*			
	A	B	C	D
Odonata (Anisoptera) larvae	0.14 (0.00–0.29)	0.10 (0.00–0.24)	0.32 (0.09–0.55)	0.04 (0.00–0.12)
<i>Ranatra linearis</i>	0.29 (0.09–0.48)	0.07 (0.00–0.18)	0.10 (0.00–0.26)	0.03 (0.00–0.10)
Consumer: <i>Proterorhinus semilunaris</i>				
<i>Bithynia tentaculata</i>	0.27 (0.03–0.51)	0.17 (0.00–0.33)	0.14 (0.00–0.34)	0.10 (0.00–0.23)
<i>Corbicula leana</i> [‡]	0.18 (0.01–0.33)	0.35 (0.15–0.58)	0.15 (0.00–0.32)	0.50 (0.29–0.71)
<i>Gammarus roeselii</i>	0.22 (0.02–0.37)	0.10 (0.00–0.23)	0.25 (0.06–0.42)	0.05 (0.00–0.14)
<i>Radix labiata</i>	0.22 (0.11–0.32)	0.15 (0.01–0.26)	0.05 (0.00–0.14)	0.15 (0.01–0.26)
<i>Sigara falleni</i>	0.11 (0.00–0.27)	0.23 (0.010–0.41)	0.40 (0.00–0.87)	0.19 (0.00–0.38)

*A: Post (2002) (Established mean trophic fractionation based on a literature review); B: McCutchan et al. (2003) (Fractionation in muscle tissue); C: McCutchan et al. (2003) (Aquatic consumers); D: Caut et al. (2009) (Based on a selection of data of fractionation in muscle tissue of fish).

[‡]: *Corbicula leana* formerly identified as *Corbicula fluminea* (see: Morhun et al. 2022)

2023a, b; Van Kessel et al. 2013). Both invaders do not dominate the benthic fish community and are considered moderately abundant, similar to the native *B. barbatus* (Binnendijk et al. 2017). *Cobitis taenia* and *G. gobio* are considered highly abundant. The mean trophic levels of the five benthic fish species ranged between 2.6 and 3.0, indicating that these benthic species are all secondary consumers (i.e., carnivores that mostly eat herbivores). The analyses demonstrated that *M. bipartitus* predominantly consumed native and alien macroinvertebrate species. The larvae of non-biting midges (Chironomidae) and dragonflies (Anisoptera), two mollusk species and a gammarid were the most important dietary items. This is in line with the results of a diet study on the closely related Oriental weatherfish *Misgurnus anguillicaudatus* (Urquhart and Koetsier 2014). No such studies have yet been conducted for *M. bipartitus*. Based on stomach analyses, it was determined that chironomid larvae, dragonfly or damselfly larvae (Odonata), mollusks, and detritus were the most frequently consumed prey of *M. anguillicaudatus*. Another stomach analysis study of *M. anguillicaudatus* also demonstrated that chironomid larvae and mollusks were important dietary items (Keller and Lake 2007). However, both studies found that worms (Oligochaeta) and water fleas (Cladocera) were also relatively important dietary items of *M. anguillicaudatus* (Keller and Lake 2007; Urquhart and Koetsier 2014). During our sampling, we did not catch Oligochaeta or Cladocera. No species belonging to these taxonomic groups were also found during previous macrofauna sampling occasions (Basten et al. 2010). The fauna sampling by dipnets with a 1 mm mesh size was probably less effective for some taxonomic groups of benthic invertebrates and zooplankton. For instance, more taxa of benthic Oligochaeta and Chironomidae can be expected in a nutrient-rich lowland stream such as the Tungelroyse beek (Redeke 1948; Tolkamp 1980). Therefore, in addition to the use of a fine-meshed dip net, a core sampler and plankton net are also recommended in future studies. Nevertheless, the information on the trophic levels of the benthic fish species shows that the examined species predominantly prey on macroinvertebrates.

Combining results of multiple sampling techniques with novel approaches for dietary analyses (e.g., mixing models, eDNA from intestines, food experiments) will reduce uncertainty in the results of future studies on the diet of benthic fish species in the Tungalroyse beek. *Proterorhinus semilunaris* consumes native and alien macroinvertebrates. A water boatman, a gammarid species, and three mollusk species were identified as the most important dietary items. Stomach analyses demonstrated that mollusks and gammarids can be important dietary items of *P. semilunaris* (Vašek et al. 2014; Všeticková et al. 2014), but also revealed that chironomid larvae were the main food sources of this species (Adámek et al. 2010; Vašek et al. 2014; Všeticková et al. 2014; Endrizalová et al. 2020). The latter is not the case in our study, despite the presence of chironomid larvae in the brook. This may indicate niche differentiation between various populations of *P. semilunaris* (see section Niche differentiation).

A comparison of the Bayesian standard ellipses shows a significant isotopic niche space overlap between the alien and native fish species. Based on their isotopic niche overlap, potential competitive interactions can be expected between *M. bipartitus* and *G. gobio* (91.9% overlap), and to a lesser extent with *B. barbatula* (37.1% overlap) and *C. taenia* (1.1% overlap). Results of Bayesian mixing models also demonstrate that partial food overlap between *M. bipartitus* and *G. gobio* is likely. Out of 28 modeled dietary items, chironomid larvae were most important for both fish species. Furthermore, they feed on larvae of Odonata (dragonflies and damselflies), the faucet snail *Bithynia tentaculata*, and the water stick insect *Ranatra linearis*. These macroinvertebrates with a relatively large body size are associated with nutrient-rich lowland brooks and mainly occur in submerged aquatic vegetation (Redeke 1948; Verdonschot et al. 1992). Potential competitive interactions with respect to food can also be expected between the alien *P. semilunaris* and native *B. barbatula* (91.2% overlap), *C. taenia* (66.1% overlap), and to a lesser extent *G. gobio* (37.4% overlap). Results of Bayesian mixing models also indicate food overlap between *P. semilunaris*, *B. barbatula*, and *C. taenia*. Out of 28 modeled dietary items, two dietary items were identified as the main communal dietary items between the latter three species: *B. tentaculata* and the water boatman *Sigara falleni*. The aquatic snail *Radix labiata* was an important dietary item for *P. semilunaris* as well as for *C. taenia*.

Niche differentiation

Niche differentiation may allow species to co-exist by avoiding competition or resource partitioning. *Proterorhinus semilunaris* displays an opportunistic and plastic feeding pattern outside its native range (Pettitt-Wade et al. 2015; Nagelkerke et al. 2018; Tarkan et al. 2018, 2019). For *M. bipartitus*, this has not yet been studied. However, diet studies on the related *M. anguillicaudatus* and European weatherfish (*Misgurnus fossilis*) demonstrated that these species are opportunistic feeders (Keller and Lake 2007; Urquhart and Koetsier 2014; Norris 2015; Pyrzanowski et al. 2021). Therefore, we assume that both benthic invaders of the present study are food generalists. The isotopic niche spaces of both alien fish species were smaller than those of native benthic species. This indicates niche differentiation, promoting coexistence due to the food specialization of the invaders (Jackson and Britton 2014; Jackson et al. 2014; Tran et al. 2015; Top-Karakuş et al. 2021). The feeding areas they frequently visit may be smaller and more restricted than for the native species because of competition for space or territory (Baltz and Moyle 1993). In fact, the fish could select diet items and feeding areas avoiding natives. The degree of isotopic niche overlap between *M. bipartitus* and *P. semilunaris* was low (8.9% overlap), indicating little resource competition between the invaders. In contrast to *M. bipartitus*, *P. semilunaris* did not have chironomid larvae as an important dietary

item, whereas these larvae appeared to be the main food source in several other studies (Adámek et al. 2010; Vašek et al. 2014; Všeticková et al. 2014; Endrizalová et al. 2020). In its native range, the isotopic niche space area of *P. semilunaris* was significantly larger than co-occurring fish species (Tarkan et al. 2018). The isotopic niche overlap between *P. semilunaris*, *B. barbatula*, and *G. gobio* is in line with the results of a study based on the functional feeding traits of these species (Nagelkerke et al. 2018). They concluded that *P. semilunaris* is a generalist feeder and its trophic profile overlaps with *B. barbatula* and *G. gobio*. *Cobitis taenia* and *M. bipartitus* were not assessed. The available body of knowledge on the feeding strategies of *M. bipartitus*, *P. semilunaris* supports the coexistence of these alien species with native species is likely due to niche differentiation by a large plasticity of their feeding behavior. We cannot demonstrate a shift in resource partitioning of native species because data on the reference situation before the arrival of the alien species are not available. Moreover, we lack dietary information in time and space.

Ontogenetic changes in feeding pattern

The body size of native fish species correlated negatively in a significant way with their $\delta^{15}\text{N}$ (‰) signature (Fig. 6). This indicates that different life stages of these species prey on other food items (Smyly 1955; Copp and Vilizzi 2004). We found that aging individuals of native species were less N enriched. Larger individuals prey on larger organisms and larger species with a lower $\delta^{15}\text{N}$ (‰) signature. The N enrichment of both alien fish species did not differ for individuals with different body sizes, probably because these species are more opportunistic and plastic in their way of feeding. A shelter competition experiment demonstrated that the presence of *P. semilunaris* did not cause shifts in the habitat choice of *B. barbatula* (Van Kessel et al. 2011). The lack of competitive behavior between these species most likely resulted in their isotopic niche overlap. Moreover, it may also explain the high degree of niche overlap (99.2%) between the native *B. barbatula* and *C. taenia*. According to the Bayesian mixing models, these species also have a considerable overlap in dietary items. This is notable because previous research has shown that several life stages of both species have different habitat preferences (Copp and Vilizzi 2004).

Robustness of the results

Isotopic niche spaces were identified by determining Bayesian standard ellipses, a relatively new and proven method that has already been applied in several studies of on various aquatic invasions (e.g., Jackson et al. 2012; Mumby et al. 2018; Balzani et al. 2020; Cerveira et al. 2021). The imperfections in the sampling of benthic macrofauna and zooplankton do not affect our results on niche widths, since these 95% confidence interval standard ellipse areas are determined based on the isotopic signatures of the benthic fish species (Jackson et al. 2011). The sample sizes used in the present study for comparing the benthic species ranged from $n = 14$ to $n = 34$. It is recommended to use the corrected standard ellipse area (SEAc) value if sample sizes are small ($n < 30$) or unequal (Jackson et al. 2011). SEAc results in a slightly large ellipse but the shape is similar to that of the regular SEA and is evenly reliable. Simulations of the Bayesian model with smaller sample sizes than those used in this study (e.g., $n = 3$, Ryan et al. 2013) can also result in robust SEAc values (Jackson et al. 2011). The use of Bayesian mixing models is a valuable addition to comparing Bayesian standard ellipses since they yield robust estimates of the diet (Parnell et al. 2010; Jackson et al. 2012). It has been demonstrated that mixing models even yield better estimates of prey identification and quantification than regular stomach

analysis (Koester et al. 2022). However, this study only focused on isotopic niches of benthic fishes. Hutchinson (1957) defined a niche as the set of biotic and abiotic conditions in which a species is able to persist and maintain stable populations over time. A niche is not fixed but can change over time due to changes in the environment or interactions with other species. This denotes a limitation of the present study as it did not examine prey abundance or foraging strategies and habitat use of different benthic fish species during the day and night, and over seasons. Diet preferences of benthic fish can be seasonal (e.g., Smyly 1955; Copp and Vilizzi 2004; Adámek et al. 2010). This could mean that there is a seasonal effect on the trophic niche overlap of co-occurring fish species. The isotopic signature measured from fish sampled in October is that of a period of high food resource richness. The trophic niches overlap may be different during periods with lower resource richness. Therefore, follow-up research should focus on unraveling seasonal variability of food niche overlap between species. Moreover, analyses of stable isotopes in this study concern a snapshot in the time of year, but also in the colonization process. To better understand the colonization process of *M. bipartitus* and *P. semilunaris*, it is also recommended that analyses be repeated at a later time, with this study serving as a baseline.

Risks of the recent invaders

The use of stable isotope analyses can be a reliable approach to quickly assess the potential impacts of invaders on native species and their environment (Balzani and Haubrock 2022). Here, we use this technique to predict the impact of two benthic invaders on three native species in a lowland brook. Lowland rivers and brooks in western Europe are usually characterized by high nutrient contents (Hering et al. 2010; De Klein and Koelmans 2011), which is also the case for the brook investigated in this study (Basten et al. 2010). Due to the high nutrient load in this brook, it is likely that food resources are not limiting the abundance of benthic fish species (Giller 1984). Therefore, the coexistence of sympatric native and alien benthic fish species is a plausible scenario.

There is evidence that both alien species are expanding their geographical range in Western Europe (Cammaerts et al. 2012; Manné et al. 2013; Verreycken 2021). In 2012, the first *M. bipartitus* population in the Netherlands was found in the brook Tungelroyse beek (Van Kessel et al. 2013). In subsequent years, the population continued to expand in this brook (Binnendijk et al. 2017). In 2020, a specimen was found in a 12 km downstream tributary of the Meuse River (Binnendijk 2020; NDFFF 2023a). It is likely that the *M. bipartitus* population will further expand and will utilize the Meuse River as a dispersal corridor to colonize other tributaries, as *P. semilunaris* did (Cammaerts et al. 2012; Manné et al. 2013). This makes it likely that habitats of the native *M. fossilis* will be invaded in the long run. In addition to risks of hybridization and disease transmission (Wanzenböck et al. 2021), resource competition may pose a significant threat to *M. fossilis* populations. *Proterorhinus semilunaris*, first found in the Netherlands in 2002, has become a widespread species (NDFFF 2023b). Given the range expansion of both alien species, it is possible that they will eventually colonize systems with fewer food resources where competition may eventually lead to the displacement of native benthic fish species.

Ethics and permits

The project was approved by the Radboud University Animal Experiment Committee and was performed under animal experimentation WOD license No. TRC/NVWA/2014/1558. An Inland Fisheries Regulation Exemption was in place for

use of electrofishing: (i.e., Ontheffing Reglement voor de Binnenvisserij, document 61165). The Water board Limburg granted permission to collect fish for the present study.

Data sharing

Source data for this study are available from: <https://doi.org/10.5281/zenodo.10100562>.

Funding declaration

The study was financed by the Radboud University, Nijmegen. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author contributions

Research Conceptualization: PL, GvdV, RSEWL; Fieldwork and lab analyses: PL, ROW, GvdV; Data analyses and interpretation: PL, GvdV, RSEWL; Draft manuscript preparation: PL. All authors reviewed and edited the results and approved the final version of the manuscript.

Acknowledgements

We would like to thank four anonymous reviewers for their critical review of an earlier version of this paper and suggestions for improvement of the text. We also thank Niels de Nijs and Joost Merjenburgh for their assistance during the sampling, Niels Eimers for help with the identification of aquatic macrophytes, Sebastian Krosse and Paul van der Ven of the General Instrumentation Laboratory of Radboud University for their help with pretreatment of the samples and the elemental analyses, Yvon Verstijnen for helping with Bayesian mixing model analyses, and Paul van Hoof for allowing us to use his photos of benthic fish species in Figure 6.

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