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# Shared Histories of Co-evolution May Affect Trophic Interactions in a Freshwater Community Dominated by Alien Species

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Interactions occurring between species in multiple invaded freshwater ecosystems are often difficult to observe and study. Studies on invasive alien species typically focus on single species, and, when the species community is exclusively composed of alien species, their interactions, eventual facilitation and hindering processes are very scarcely assessed. To investigate such a community, the species community in the Arno River (Tuscany, Italy) was examined as a model system using the combined approach of stable isotopes and dietary analyses. Established alien species have formed a pyramid shaped community with the European catfish *Silurus glanis* and the North American channel catfish *Ictalurus punctatus* in the apex position, followed by opportunistic predatory (*Lepomis gibbosus*) and omnivore (*Alburnus alburnus*, *Cyprinus carpio*, *Barbus barbus, Pseudorasbora parva, Padogobius* sp., *Tinca tinca*) species. These species were observed to feed on a variety of primary producers (*Myriophyllum* sp., *Potamogeton* sp., *Phragmites australis*) and consumers (aquatic insects, molluscs, *Dikerogammarus villosus*, *Procambarus clarkii*, *Palaemon antennarius*, tadpoles). Remarkably, the channel catfish *Ictalurus punctatus* showed an ontogenetic niche shift, with juveniles occupying a slightly higher trophic position than mature individuals. *Pseudorasbora parva* and *A. alburnus* showed a strong niche overlap, with the former having a wider niche. Such wide niches were also found for the invasive crustaceans *D. villosus* and *P. clarkii*. Outgoing from our findings, we suggest that life-history and geographic origin play a role in determining competition and interaction type among alien species, with species from the same geographic area showing a lower potential to compete than species from different areas, hence affecting their potential impact on native species.

**Keywords: stable isotopes, stomach contents, community structure, mixing models, feeding ecology, interactions**

# INTRODUCTION

The invasive potential as well as distribution of an alien species depend on various abiotic and biotic factors such as its life-history (Kolar and Lodge, 2001; Keller et al., 2007; Strayer, 2010; Blackburn et al., 2011), predator-prey dynamics, adaptability as well as interactions with other species (Ricciardi, 2003; Strayer et al., 2006; Ricciardi et al., 2013). Subsequent, the introduction and successive dispersal of alien species change the identity of entire species communities (Seebens et al., 2017). When multiple invasive alien species are dominating one freshwater ecosystem, they tend to occupy different trophic levels (Gamradt and Kats, 1996; Kiesecker and Blaustein, 1998), but the presence of invasive species interacting across trophic levels increases the already-existing complex threat through direct and indirect effects (Peckarsky and McIntosh, 1998; Huxel et al., 2002; Strayer, 2010), as the prevalence of omnivores (Pringle and Hamazaki, 1998), and interactions among predators (Sih et al., 1998) are unforeseeable. However, such complex interactions among invasive species are not well understood (Parker et al., 1999; Huxel et al., 2002; Strayer, 2010) but, as described in the "invasional meltdown theory," can enhance the chance of a species to successfully establish itself and increase or add further impacts on the recipient environment (Simberloff and Von Holle, 1999; Simberloff, 2006). Otherwise, these interactions can lower the impact of predatory invasive species on native species in certain cases (Soluk and Collins, 1988a,b; Soluk, 1993; Rosenheim, 1998; Bissattini et al., 2018). Despite the utmost importance of such studies on multiple invaded ecosystems for practical management and control efforts (Polis and Strong, 1996; Rosenheim, 1998), they are still scarce (but see Johnson et al., 2009; Gherardi et al., 2011; Haubrock et al., 2019a).

The use of Stable Isotopes Analyses (SIA) enables the study of invasive species and their interactions with native biota (Vander Zanden et al., 1999; Balzani et al., 2016). In particular, SIA proved to be a useful tool in investigating the impacts of alien fish (Cucherousset et al., 2012; Britton et al., 2018). Using SIA for carbon (C) and nitrogen (N), long-term and time-mediated information of a community trophic structure can be obtained (Boecklen et al., 2011; Layman et al., 2012; Middelburg, 2014), trophic levels can be estimated (Post, 2002), and feeding ecological niches finely quantified (Newsome et al., 2007). While C signatures identify the major energy sources, N signatures relate to the trophic position of a consumer within a food web (Fry, 2006; Layman et al., 2012). This relationship relies on predictable changes in the isotopic signal from prey to consumer, being enriched by 1‰ for C and by 2.5-5‰ for N between consecutive trophic levels (Post, 2002; Vanderklift and Ponsard, 2003). Combined with the analysis of dietary contents (DA), which provide a direct but short temporal insight into the feeding habit of a species, relationships among species can be investigated with a considerably higher resolution.

Aquatic ecosystems, especially in the Mediterranean areas, have been subjected to successive invasions (Dumont et al., 2004; Marr et al., 2010). For instance, Italian freshwaters, with an ichthyofauna composed of 48 native and 41 established introduced and further 15 introduced but not yet established species, have been imperiled by invasions (Nocita et al., 2017). Many of these species are notoriously invasive, like the European catfish *Silurus glanis* (Copp et al., 2007, 2009; Boulêtreau et al., 2018), which has become the object of various management efforts (Gualtieri and Mecatti, 2005; Cucherousset et al., 2018), while others like the channel catfish *Ictalurus punctatus* have received minor attention, with only few recent studies addressing its potential impacts (Haubrock et al., 2018a,b). In the Arno River in Tuscany, species introductions occurred repeatedly, as in the case of *Blicca bjoerkna*, *Rhodeus sericeus*, *I. punctatus*, and *S. glanis* (Nocita, 2002, 2007), that are now established (Nocita and Zerunian, 2007). Moreover, anthropogenic effects combined with introductions have caused the local extinction of native predators (e.g., *Esox lucius*; Nocita and Zerunian, 2007). Therefore, the Arno River is a peculiar ecosystem, because almost no native fish species is currently present (Nocita, 2002), and it is thus characterized by unique species composition, with so far unknown interactions and diverse introduction histories. Moreover, several of the species present in the Arno river (e.g., *P. clarkii*, *S. glanis*, *D. villosus*, *G. chinensis*, *P. antipodarum*, *P. parva*, *G. holbrooki*; Gherardi et al., 2008; Nocita and Lenuzza, 2016) are listed among the worst invasive species and other comparable lists of high-profile invasive species (Vilà et al., 2009; Nentwig et al., 2018). It can be considered as a unique melting pot for alien species from Asia and North America, mainly voluntarily introduced for sport fishing and commercial fishing purposes (Nocita, 2002; Vejrík et al., 2019). Consequently, manifold interactions that can only be observed in such an invaded range are of great importance from both a theoretic and management point of view.

We, thus, hypothesize that species interactions among and across trophic levels and, especially top-down impacts, are affected not only by differing life-histories but also a shared history of co-evolution of the invading species, with invasive alien species coming from different areas being more likely to compete due to the lack of the latter.

Hence, the aims of this work were to: (i) combine SIA and DA to investigate feeding preferences and impacts of alien species in the investigated community mainly composed by alien species; (ii) compare species' trophic niches and niche overlap to infer the potential degree of feeding competition among species; (iii) unravel important functional roles of organisms (as pointed out by (Bissattini and Vignoli, 2017) for *P. clarkii*, linking terrestrial and aquatic energy sources), and (iv) discuss results in regard to invasive species' origins as a potential factor determining the outcome of species interactions.

# MATERIALS AND METHODS

## Study Site and Sampling

The Arno River is with a length of 241 km and a drainage of more than 8.200 km² as well as an effluent flow of 100 m²/s the second most important river in Central Italy (Nocita and Zerunian, 2007). Its discharge regime is typical of Mediterranean rivers showing extreme flow reductions during summer. It flows through the major cities of Florence and Pisa and is especially within Florence divided by various weirs and characterized by a

high density of non-native aquatic vegetation during spring and early summer. During summer, the water of the Arno River in the inner-city section of Florence can reach mean temperatures of 29.6°C (min: 27.2, max: 32.4; data from 2016 to 2018 http://www.arpat.toscana.it). The historic use and cultural value have led to high anthropogenic stress on communities. Indeed, the aquatic community consists of various alien species while only a minority of them is considered native (Nocita, 2002). However, a detailed list of alien species has not been published so far (pers. comm. Gianna Innocenti).

Sampling was conducted from April to June 2018, in a very anthropogenically disturbed part of the inner-city (Florence) section of the Arno River which is highly used for recreational angling (43.765606, 11.268234; DATUM WGS84). The specific period was chosen, as these months are the ideal activity period for several fish species (samples were taken approximately 2 month after the inactive winter period, thus after species had resumed their feeding activity). Moreover, the effect of angling as a stressor that could interfere with the species trophic positioning through the input of "unusual food sources" is limited due to low angling activity (pers. Comm, Thomas Bussatto). To collect the several species, a combined approach of several methods was applied. Fish were caught with standard fishing rods and the help of local fishermen using a variety of baits and fishing techniques. Macroinvertebrates and frog tadpoles were collected using funnel traps and hand nets. Sampled species were collected under the consideration of an even spatial distribution across the sampled stretch of the river ecosystem without bias toward either section, end or distance from riverbank (Willson et al., 2010). Samples of the local aquatic vegetation were also taken as they represent the baseline of the ecosystem. Sampled specimens were immediately euthanized (fish: gills cutting; macroinvertebrates: freezing), stored on ice during transport and then preserved in the freezer at −20°C until further processing. Overall, 232 specimens belonging to nine fish species, 71 invertebrates from at least six species, four green frog tadpoles, and thirteen samples from three different plant species (two hydrophytes: *Potamogeton* sp. and *Myriophyllum* sp., and one helophyte: *Phragmites australis*) were collected. Due to the rarity of both *Tinca tinca* and *Barbus barbus* in the study site, only one sample of each was caught and included in the dataset to roughly indicate these species positioning in the community. However, these samples were not used for any further statistical analysis. The sampling was authorized by the Tuscan Region ("Autorizzazione alla pesca scientifica, Regione Toscana").

## Sample Preparation and Analyses

For all fish species, total length (TL) was measured to infer age, as fish growth is generally continuous, presenting a link between size and age (Hopkins, 1992). Resultant, over TL; the age can be inferred and used to identify fish maturity, which can in turn be used to investigate behavioral differences (Haubrock et al., 2019b). Additionally, weight (W), and, if distinguishable, also sex were recorded. TL of fish and cephalothorax length (CTL) of crayfish were measured with a caliper and W was measured using an electronic balance. In the laboratory, stomach contents were extracted, and prey items identified to the lowest

possible taxon under a stereomicroscope (Sansoni, 1998; Schultz, 2010). We estimated number of stomachs containing each food item in relation to total number of full stomachs (frequency of occurrence, F%) and number of individuals of each food item with respect to the total number of individuals (number of occurrence, N%) for each food item in each respective species (Haubrock et al., 2019b). Despite the information DA provide on the direct feeding activity, they are frequently used to supplement stable isotope analyses as priors due to their often differing results (Pacioglu et al., 2019). However, from all species analyzed, several had empty stomachs (*S. glanis*: 20/39; *A. alburnus*: 2/10; *P. parva*: 4/10; *L. gibbosus*: 7/20). For isotopic analyses, muscle tissue was cleaned of fat, skin, scales, bones and carapace (for crayfish). For plants, different leaves were cut-off from the same individual and pooled in one sample to account for eventual differences. Due to their small size, insects, little crustaceans and tadpoles were treated as whole samples while, for molluscs, only the soft body tissue was analyzed without the shell. Samples were placed on separated glass trays and dried for 48 h at 60°C. Once dry, all samples were grinded into fine powder with an agate mortar and pestle. For each sample, two replicates of 0.20–0.30 mg for animals and 1.00–1.10 mg for plants, were weighed on a Mettler Toledo AG245 microscale and enclosed in a tin capsule to be analyzed in continuous flow—isotope ratio mass spectrometry (CF-IRMS) with a Thermo FlashEA 1112 elemental analyser and a Thermo Finnigan Delta Plus isotope ratio mass spectrometer at the Istituto di Geologia Ambientale e Geoingegneria (IGAG) of the National Research Council (CNR) in Montelibretti, Rome. Isotope compositions were expressed as ‰ with the δ notation (based on $\delta^{13}C$ or $\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1000$, where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratios]. Results were referred to Vienna Pee Dee Belemnite for carbon and to atmospheric $N_2$ for nitrogen, with laboratory standards calibrated on IAEA international standards: N1, N2, and USGS25 for nitrogen and CH6, CH7, and USGS24 for carbon.

## Statistical Analyses

The arithmetic mean ± SE was calculated, and eventual correlations between $\delta^{15}N$ and $\delta^{13}C$ values and TL, CTL and W measures were tested for each species using a linear model. The trophic position (TP) of each species was estimated using the R package "tRophicPosition," which incorporates a Bayesian model utilizing up to two baselines (Quezada-Romegialli et al., 2018). Hence, we included the combined plants (*Myriophyllum* sp., *Potamogeton* sp., *Phragmites australis*) as first, and molluscs (*Gyraulus chinensis*) as second baseline (n.adapt = 100,000; n.iter = 100,000; burnin = 10,000; n.chains = 5). These two groups were chosen due to the wide diet preferences known for alien fish species (Musil and Adámek, 2007; Almeida et al., 2009; Xu et al., 2011; Ribeiro and Leunda, 2012; Haubrock et al., 2019a,b).

To quantify intra-specific and community niche width, Layman metrics (Layman et al., 2007) were calculated with the R package "SIAR" (Stable Isotope Analysis in R; Parnell et al., 2010). Additionally, the corrected standard ellipse areas (SEAc, considering 40% of data plots) and SEAb (Bayesian standard ellipse areas, considering 95% of data plots) were calculated for all fish species with $n \geq 5$ as well as *Procambarus clarkii* using

**TABLE 1 |** Frequency of occurrence (%F), abundance (%N), and prominence value (PV) of food items analyzed for juvenile and mature *Ictalurus punctatus*, juvenile *Silurus glanis*.

| Prey items | *Ictalurus punctatus* (Immature) | | *Ictalurus punctatus* (Mature) | | *Ictalurus punctatus* (Total) | | *Silurus glanis* (Immature) | | *Lepomis gibbosus* | | *Pseudorasbora parva* | | *Alburnus alburnus* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [F%] | [N%] | [F%] | [N%] | [F%] | [N%] | [F%] | [N%] | [F%] | [N%] | [F%] | [N%] | [F%] | [N%] |
| Detritus | 0.30 | 0.08 | 0.25 | 0.04 | 0.30 | 0.06 | 0.10 | 0.09 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Plants | 0.45 | 0.09 | 0.60 | 0.14 | 0.45 | 0.12 | 0.13 | 0.11 | 0.00 | 0.00 | 0.10 | 0.50 | 1.00 | 1.00 |
| Detergent | 0.20 | 0.08 | 0.05 | 0.01 | 0.20 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Palaemon antennarius* | 0.25 | 0.18 | 0.15 | 0.04 | 0.25 | 0.10 | 0.03 | 0.02 | 0.05 | 0.01 | 0.05 | 0.25 | 0.00 | 0.00 |
| *Dikerogammarus villosus* | 0.18 | 0.14 | 0.20 | 0.03 | 0.18 | 0.08 | 0.05 | 0.07 | 0.13 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Procambarus clarkii* | 0.10 | 0.01 | 0.15 | 0.03 | 0.10 | 0.02 | 0.00 | 0.00 | 0.13 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 |
| Unid. Crustaceans | 0.08 | 0.14 | 0.00 | 0.00 | 0.08 | 0.06 | 0.03 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Terr. Insects | 0.43 | 0.16 | 0.45 | 0.29 | 0.43 | 0.23 | 0.00 | 0.00 | 0.00 | 0.00 | 0.05 | 0.25 | 0.00 | 0.00 |
| Aq. Insects | 0.13 | 0.00 | 0.25 | 0.06 | 0.13 | 0.03 | 0.03 | 0.02 | 0.75 | 0.90 | 0.00 | 0.00 | 0.00 | 0.00 |
| Hirudinea | 0.03 | 0.00 | 0.05 | 0.01 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Fish larvae | 0.08 | 0.02 | 0.05 | 0.01 | 0.08 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Unid. Fish | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.03 | 0.02 | 0.25 | 0.05 | 0.00 | 0.00 | 0.00 | 0.00 |
| Small cyprinids | 0.25 | 0.05 | 0.30 | 0.08 | 0.25 | 0.06 | 0.18 | 0.16 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Large cyprinids | 0.03 | 0.00 | 0.05 | 0.01 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Ictalurus punctatus* | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.08 | 0.09 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Molluscs | 0.05 | 0.00 | 0.10 | 0.04 | 0.05 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Small mammals | 0.03 | 0.00 | 0.05 | 0.01 | 0.03 | 0.00 | 0.03 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Small birds | 0.08 | 0.01 | 0.10 | 0.02 | 0.08 | 0.01 | 0.03 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Testudines | 0.03 | 0.00 | 0.05 | 0.01 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Fishing baits | 0.23 | 0.03 | 0.35 | 0.17 | 0.23 | 0.11 | 0.03 | 0.34 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

*Lepomis gibbosus, Pseudorasbora parva, and Alburnus alburnus; "0.00" indicates that the specific prey items in the fish's diet were considerably low; "na" indicates that respective prey item was not present.*

the R package "SIBER" (Stable Isotope Bayesian Ellipses in R; Jackson et al., 2011).

The application of scatter plot for $\delta^{15}$N vs. $\delta^{13}$C of consumer tissues and food sources enabled the determination of probable prey sources and combinations of prey contributing to the diet of predators (Phillips and Gregg, 2003). Dietary analyses (DA) for catfish species (juvenile and mature *I. punctatus* as well as *S. glanis*) were previously performed (Haubrock et al., 2019b), while DA for *Lepomis gibbosus*, *Pseudorasbora parva*, and *Alburnus alburnus* were conducted following the same protocol (**Table 1**). Stable isotope mixing models (SIMM; R package "SIMMr") were applied for all fish and crayfish without consideration of priors but considering potentially predated items, i.e., a predation on lower trophic levels. Thus, it was investigated how sampled species contribute to the isotopic signatures of each other, indicating the scenario with the highest probability (Parnell et al., 2013). Additionally, the same analyses were performed again for *L. gibbosus*, both life-stages of *I. punctatus* and *S. glanis* with results from DA as priors for the analyses (i.e., considering: the five most consumed prey items and then their number of occurrence N%; 4 prey items were used for *L. gibbosus*; **Table 1**). Priors from DA were not included into the mixing models for *P. parva* nor *A. alburnus* due to the low number of stomachs available ($n = 10$ for either species) of which the majority was empty (*P. parva* = 6; *A. alburnus* = 8). Prior based analyses were conducted with the R package "SIAR" (Parnell and Jackson, 2013). Results are presented as the average percent values with the possible range percentage for each prey item. With these, the trophic web of the studied ecosystem was reconstructed. Furthermore, using the software PRIMER (Clarke and Warwick, 2001) a Multi-Dimensional Scaling plot (MDS) was drawn and a Permutational Analysis of Variance (PERMANOVA; Resemblance: Euclidean distance; Sums of squares type: Type III (partial); Fixed effects sum to zero for mixed terms; Permutation method: Unrestricted permutation of raw data; Levels: 15; Number of permutations: 9999) was performed to test for significant differences in $\delta^{15}$N and $\delta^{13}$C among species and to eventually determine niche overlaps. Lastly, we compared the results among species to discuss the effect of an alien species origin in respect to indicated trophic interactions.

## RESULTS

Overall, 9 invasive but no native fish species were sampled. There were complimented by 2 invasive and 1 native crustacean as well as 1 invasive mollusc. Additionally, 2 native insect, 1 native amphibian and 3 invasive plant species were collected (**Table 2**).

From these, some species were under-sampled due to their rarity (i.e., the locally alien species *Tinca tinca* and *Barbus barbus*) or status as endangered (i.e., *Padogobius* sp.), while only a minimum of *Cyprinus carpio* and no mature *S. glanis* (>80 cm) individuals were sampled to meet the demands of local anglers. Additionally, the alien omnivore *Squalius cephalus*, which is believed to be rare or locally extinct, was not sampled. The abundant invasive pond slider *Trachemys scripta* as well as the occasionally found alligator snapping turtle *Macrochelys*

**TABLE 2 |** Morphological measures (mean ± SE), sex ratio (calculated as ratio of females, f. on males, m) and number of all sampled specimens. Length measured as total length (TL) for fishes and as cephalothorax length (CTL) for crayfish; $n =$ number of specimens caught.

| Species | Length [cm] | Weight [g] | Sex ratio [f:m] | $n$ |
|---|---|---|---|---|
| **Fish** | | | | |
| *Silurus glanis* | 27.1 ± 1.1 | 122.8 ± 14.4 | 1:1 | 39 |
| *Ictalurus punctatus* | 33.8 ± 2.2 | 538.5 ± 106.3 | 1:1 | 40 |
| *Cyprinus carpio* | 31.2 ± 9.9 | 584.3 ± 506.7 | 3:1 | 4 |
| *Tinca tinca* | 9.8 | 11 | – | 1 |
| *Barbus barbus* | 17 | 36 | – | 1 |
| *Padogobius* sp. | 4.4 ± 0.1 | 2.3 ± 0.5 | – | 4 |
| *Lepomis gibbosus* | 9.1 ± 2.9 | 20.0 ± 16.6 | 1:1 | 20 |
| *Pseudorasbora parva* | 8.0 ± 1.3 | 4.8 ± 2.3 | – | 16 |
| *Alburnus alburnus* | 9.4 ± 1.0 | 7.3 ± 2.5 | – | 20 |
| **Crustaceans** | | | | |
| *Procambarus clarkii* | 6.5 ±2.4 | 26.2 ± 1.7 | 1:1 | 10 |
| *Palaemon antennarius* | | | | 16 |
| *Dikerogammarus villosus* | | | | 11 |
| **Insects** | | | | |
| Odonata | | | | 15 |
| Ephemeroptera | | | | 9 |
| **Amphibians** | | | | |
| *Pelophylax* kl. *esculentus* (tadpoles) | | | | 4 |
| **Molluscs** | | | | |
| *Gyraulus chinensis* | | | | 9 |
| **Plants** | | | | |
| *Potamogeton* sp. | | | | 6 |
| *Phragmites australis* | | | | 5 |
| *Myriophyllum* sp. | | | | 2 |

*temminckii* were not sampled as the sampling method was not suited to collect them. Moreover, only few macroinvertebrates were found and collected. Detailed results from DA are listed in **Table 1** and have been discussed previously for *I. punctatus* and juvenile *S. glanis* (Haubrock et al., 2019b). While the number of stomach contents analyzed for *P. parva* and *A. alburnus* were too low to be analyzed in depth or to be used as prior for subsequent mixing models, *L. gibbosus* showed a primary feeding preference for aquatic insects and secondarily for crustaceans (**Table 1**).

Statistically significant negative correlations were found between TL and $\delta^{13}$C for *S. glanis* ($r_s = -0.512$; $p = 0.001$; $n = 39$), *I. punctatus* ($r_s = -0.665$; $p < 0.001$; $n = 40$) as well as *P. clarkii* ($r_s = -0.693$; $p = 0.026$; $n = 10$). A statistically significant positive correlation between TL and $\delta^{13}$C were identified for *Pseudorasbora parva* ($r_s = 0.567$; $p = 0.022$; $n = 16$). Additionally, a statistically significant positive correlation between TL and $\delta^{15}$N was found for *L. gibbosus* ($r_s = 0.917$; $p < 0.001$; $n = 20$) (**Supplement 1**).

Following the assumption of an isotopic enrichment of $\delta^{15}$N by about 3.4‰ (Post, 2002), the entire isotopic community spanned over approximately three to four trophic levels (**Figure 1**). Considering as baseline both molluscs and plants
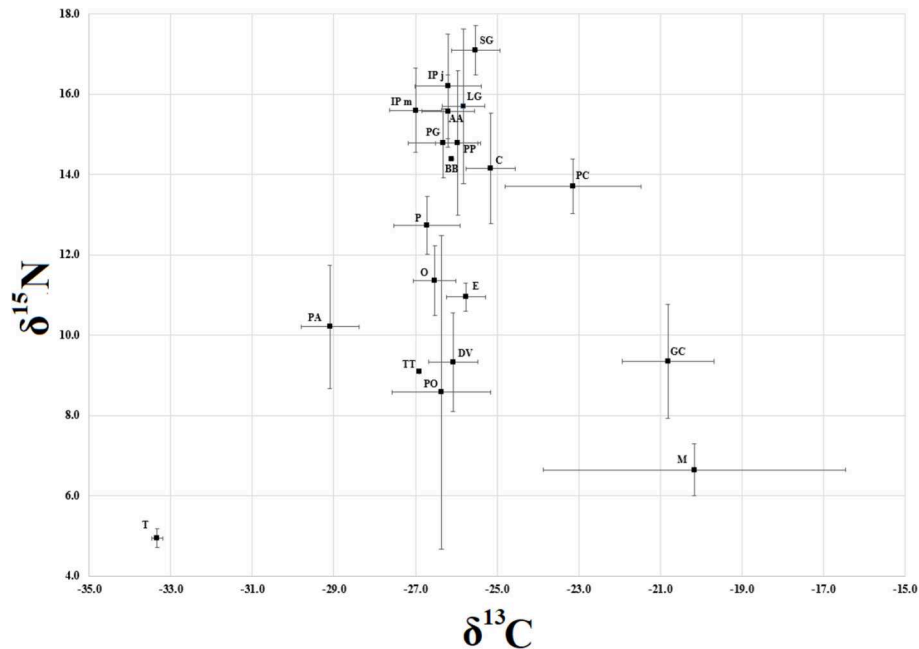
**FIGURE 1** | Isotopic space scatterplot of the community. Species IDs: SG, *Silurus glanis*; IPj, *Ictalurus punctatus* (juveniles); IPm, *Ictalurus punctatus* (matures); LG, *Lepomis gibbosus*; AA, *Alburnus alburnus*; PG, *Padogobius* sp.; PP, *Pseudorasbora parva*; BB, *Barbus barbus*; C, *Cyprinus carpio*; PC, *Procambarus clarkii*; P, *Palaemon antennarius*; O, Odonata; E, Ephemeroptera; DV, *Dikerogammarus villosus*; GC, *Gyraulus chinensis*; PA, *Phragmites australis*; T, tadpoles (*Pelophylax* kl. *esculentus*); TT, *Tinca tinca*; M, *Myriophyllum* sp.; PO, *Potamogeton* sp. Each species is represented as mean (centroid), with bars representing standard deviation.

provided a well-structured hierarchy. The 4th apex level was constituted by *S. glanis* and both life stages of *I. punctatus*; the 3rd level by *C. carpio*, *B. barbus*, *Padogobius* sp., *L. gibbosus*, *P. parva*, *A. alburnus*, *P. clarkii*, and *P. antennarius*; the 2nd level by *T. tinca*, *D. villosus*, tadpoles, Odonata, and Ephemeroptera larvae.

Layman's metrics are shown in **Table 3**. Among fish species, the greatest N range (NR) was found for *P. parva* (7.51), followed by *L. gibbosus* (6.38) and adults of *I. punctatus* (4.60), while the narrowest values were presented by *Padogobius* sp. (1.79) and *S. glanis* (2.71). C range (CR) was largest for *A. alburnus* (3.24) and smallest for *C. carpio* (1.32). Total Area (TA, i.e., the convex hull area enclosing all individual points) was highest in *P. parva* (9.64), followed by *I. punctatus* juveniles (8.76) and *L. gibbosus* (7.81), and was narrowest in *Padogobius* sp. (0.13) and *C. carpio* (1.53). SEAc, which is less influenced by extreme values than TA, was highest in *L. gibbosus* (3.33), immediately followed by *P. parva* (3.27) then *I. punctatus* juveniles (2.20); the lowest values of SEAc were found for *Padogobius* sp. (0.17), *S. glanis* (0.87) and *C. carpio* (1.72). However, the small metrics for *Padogobius* sp. and *C. carpio* have to be considered with caution, due to the small number of collected individuals ($n = 4$ for both species). Among crustaceans, *D. villosus* had the highest NR (4.40), followed by *Palaemon* (2.11) and *P. clarkii* (2.07), while CR followed the exactly opposite hierarchy: *P. clarkii* = 3.96, *Palaemon* = 2.95 and *D. villousus* = 1.90. TA was comparable between *P. clarkii* and *D. villosus* (4.60 and 4.49, respectively), while smaller in *Palaemon* (3.38). In contrast, SEAc was highest for *P. clarkii*

(3.59), followed by *D. villosus* (2.36), then *Palaemon* (1.39). Finally, tadpoles showed the lowest Layman's metrics of the whole community.

Considerable SEAc overlap was identified between juvenile *I. punctatus* and *A. alburnus* (0.50), matures *I. punctatus* and *L. gibbosus* (0.47), *L. gibbosus* and *P. parva* (0.49), and *A. alburnus* and *P. parva* (1.33). Overlap was generally low among all other fish species. *P. clarkii* showed particularly low overlap values with any fish species. The estimated SEAb overlaps indicated considerable overlap between life stages of *I. punctatus* (0.42), *P. parva* and *L. gibbosus* (0.59), and *A. alburnus* and *P. parva* (0.46) and minor overlap values between all other species. The ellipse overlaps between *A. alburnus* and *P. parva* estimated with SEAb was considerably lower than the SEAc overlap. Lastly, while the identified SEAb overlap was low between mature *I. punctatus* and juvenile *S. glanis* (0.09), juveniles *I. punctatus* and *L. gibbosus* (0.17), SEAc overlap did not indicate any overlap at all. Similarly, low SEAb overlaps between matures *I. punctatus* and *P. parva* (0.12), juvenile *S. glanis* and *A. alburnus* (0.22), and juvenile *S. glanis* and *P. parva* (0.16) were considerably higher than the respective SEAc overlap values, all being near 0 (**Table 4**).

Results of the applied SIMM without priors (**Figure 2**) indicated that both *I. punctatus* juveniles and adults expressed a great reliance on *A. alburnus* with a higher proportion in matures compared to juveniles, which consumed other items as well. *Silurus glanis* SIMM also showed a specialized piscivorous diet, focusing mostly on the consumption of *L. gibbosus*. In

**TABLE 3 |** Mean values of isotopic signatures, estimated trophic positions, Layman's metrics.

| | SPECIES | TP | $\delta^{15}$N (‰) | $\delta^{13}$C (‰) | NR | CR | TA | CD | MNND | SDNND | SEAc | n |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fish | Ictalurus punctatus (Immature) | 4.096 | 16.2 | −26.2 | 4.12 | 2.96 | 8.76 | 0.94 | 0.46 | 0.49 | 2.20 | 20 |
| | Ictalurus punctatus (Mature) | 4.006 | 15.6 | −27.0 | 4.60 | 1.96 | 5.39 | 1.06 | 0.43 | 0.25 | 1.84 | 20 |
| | Silurus glanis (Immature) | 4.366 | 17.1 | −25.5 | 2.71 | 2.82 | 4.10 | 0.70 | 0.19 | 0.17 | 0.87 | 39 |
| | Cyprinus carpio | 3.481 | 14.1 | −25.2 | 3.11 | 1.32 | 1.53 | 0.93 | 0.89 | 0.65 | 1.72 | 4 |
| | Tinca tinca[†] | 2.267 | 9.1 | −26.9 | na | 1 | | | | | | |
| | Barbus barbus[†] | 3.501 | 14.4 | −26.1 | na | 1 | | | | | | |
| | Padogobius sp. | 3.682 | 14.8 | −26.3 | 1.79 | 1.76 | 0.13 | 0.80 | 0.45 | 0.46 | 0.17 | 4 |
| | Lepomis gibbosus | 3.956 | 15.7 | −25.8 | 6.38 | 2.36 | 7.81 | 1.67 | 0.44 | 0.24 | 3.33 | 20 |
| | Pseudorasbora parva | 3.700 | 14.8 | −26.0 | 7.51 | 2.13 | 9.64 | 1.41 | 0.69 | 0.71 | 3.27 | 16 |
| | Alburnus alburnus | 3.925 | 15.6 | −26.2 | 3.01 | 3.24 | 5.27 | 0.95 | 0.37 | 0.29 | 1.80 | 20 |
| Crustacean | Procambarus clarkii | 3.217 | 13.7 | −23.1 | 2.07 | 3.96 | 4.60 | 1.58 | 0.52 | 0.26 | 3.59 | 10 |
| | Palaemon antennarius. | 3.087 | 12.7 | −26.7 | 2.11 | 2.95 | 3.38 | 0.93 | 0.30 | 0.20 | 1.39 | 16 |
| | Dikerogammarus villosus | 2.084 | 9.3 | −26.1 | 4.40 | 1.90 | 4.49 | 1.20 | 0.51 | 0.42 | 2.36 | 11 |
| Amphibian | Pelophylax kl. esculentus (tadpoles) | 2.056 | 4.9 | −33.3 | 0.58 | 0.27 | 0.05 | 0.17 | 0.18 | 0.13 | 0.07 | 4 |
| Insects | Odonata | 2.676 | 11.5 | −26.2 | 2.01 | 0.89 | 0.92 | 0.55 | 0.36 | 0.50 | 0.71 | 15 |
| | Ephemeroptera | 2.578 | 11.0 | −25.8 | 1.11 | 1.61 | 0.87 | 0.49 | 0.37 | 0.14 | 0.51 | 9 |
| Molluscs | Giraulus chinensis[‡] | na | 9.3 | −20.9 | 4.15 | 3.48 | 7.67 | 1.51 | 0.97 | 0.34 | 5.13 | 9 |
| Plants | Potamogeton sp.[‡] | na | 8.6 | −26.4 | 11.59 | 3.21 | 18.43 | 2.89 | 2.58 | 2.98 | 18.09 | 6 |
| | Phragmites australis[‡] | na | 10.2 | −29.1 | 3.65 | 1.62 | 1.79 | 1.37 | 0.92 | 0.62 | 2.35 | 5 |
| | Myriophyllum sp.[†/‡] | na | 6.6 | −20.2 | na | 2 | | | | | | |
| | Whole consumers community | na | 12.14 | 12.53 | 62.86 | 3.29 | 1.53 | 2.12 | 25.83 | 232 | | |

[†] number of samples not sufficient for the estimation of Layman's metrics; [‡] organisms used as baselines.

TP, trophic position.

n, samples number.

NR, $\delta^{15}$N range.

CR, $\delta^{13}$C range.

TA, convex hull area.

CD, mean distance to centroid.

MNND, mean nearest neighbor distance.

SDNND, standard deviation of the nearest neighbor distance.

SEAc, corrected Standard Ellipse Area.

contrast, *L. gibbosus* had a more generalist diet, based almost equally on tadpoles and various invertebrates: Ephemeroptera and Odonata larvae among insects, and *Palaemon* sp., *D. villosus* and the invasive *P. clarkii* among crustaceans. For *P. parva*, the estimated diet consisted mainly of *Palaemon* sp., followed by Ephemeroptera and then by Odonata, *D. villosus* and *P. clarkii*. The diet of *A. alburnus* is similar, but more balanced and with a marked preference for *Palaemon*. Finally, *P. clarkii* showed a preference for molluscs, followed by insects (Ephemeroptera and Odonata), *Palaemon*, plants (*Phragmites*), and tadpoles.

Mixing models refined using dietary priors (**Figure 3**) showed differing trends, highlighting a predation of *I. punctatus* juveniles on *P. antennarius*, *D. villosus*, as well as on small cyprinids (e.g., *A. alburnus*) and of *I. punctatus* (matures) on cyprinids, *P. antennarius* and *D. villosus*. As for *S. glanis*, *I. punctatus* and *D. villosus* constituted the preferred preys. Differing results were obtained for *L. gibbosus*; without numerical priors, *Palaemon*

sp. and, with priors, aquatic insects majorly contributed to its trophic level. However, SIMMs including N% priors led to an increase in the estimated proportion of contributing prey items and less variability.

The applied PERMANOVA on carbon and nitrogen signatures highlighted an overall distinction among species [$F_{(14, 202)} = 135.34$, $p = 0.001$; **Table 5**]. Nonetheless, the applied pairwise *post-hoc* tests (**Supplement 2**) indicated that some fish species had no distinct isotopic niches. In particular, overlapping niches were found between *A. alburnus* and juvenile *I. punctatus* ($p = 0.054$), *A. alburnus* and *L. gibbosus* ($p = 0.46$), *A. alburnus* and *Padogobius* sp. ($p = 0.185$), *A. alburnus* and *P. parva* ($p = 0.085$), *C. carpio* and *L. gibbosus* ($p = 0.124$), *C. carpio* and *Padogobius* sp. ($p = 0.193$), *C. carpio* and *P. parva* ($p = 0.337$), juvenile *I. punctatus* and *L. gibbosus* ($p = 0.221$), mature *I. punctatus* and *Padogobius* sp. ($p = 0.146$), *L. gibbosus* and *Padogobius* sp. ($p = 0.35$), *L. gibbosus* and *P. parva* ($p = 0.145$), *Padogobius* and *P. parva* ($p = 0.803$).

| Pairwise niche comparison | SEAb overlap | SEAc overlap |
|---|---|---|
| *Ictalurus punctatus* total—*Silurus glanis* (Matures) | 0.195 | 0.007 |
| *Ictalurus punctatus* (Immature)—*Silurus glanis* (Matures) | 0.278 | 0.224 |
| *Ictalurus punctatus* (Mature)—*Silurus glanis* (Matures) | 0.086 | 0 |
| *Ictalurus punctatus* (Immature)—*Ictalurus punctatus* (Mature) | 0.421 | 0.274 |
| *Alburnus alburnus*—*Pseudorasbora parva* | 0.462 | 1.326 |
| *Alburnus alburnus*—*Lepomis gibbosus* | 0.218 | 0.207 |
| *Pseudorasbora parva*—*Lepomis gibbosus* | 0.591 | 0.490 |
| *Ictalurus punctatus* (Immature)—*Lepomis gibbosus* | 0.167 | 0 |
| *Ictalurus punctatus* (Mature)—*Lepomis gibbosus* | 0.244 | 0.472 |
| *Ictalurus punctatus* (Immature)—*Alburnus alburnus* | 0.254 | 0.501 |
| *Ictalurus punctatus* (Immature)—*Pseudorasbora parva* | 0.168 | 0.152 |
| *Ictalurus punctatus* (Mature)—*Alburnus alburnus* | 0.187 | 0.191 |
| *Ictalurus punctatus* (Mature)—*Pseudorasbora parva* | 0.125 | 0.002 |
| *Procambarus clarkii*—*Alburnus alburnus* | 0.054 | 0 |
| *Procambarus clarkii*—*Pseudorasbora parva* | 0.057 | 0 |
| *Procambarus clarkii*—*Lepomis gibbosus* | 0.072 | 0 |
| *Procambarus clarkii*—*Ictalurus punctatus* (Immature) | 0.106 | 0 |
| *Procambarus clarkii*—*Ictalurus punctatus* (Mature) | 0.110 | 0 |
| *Procambarus clarkii*—*Silurus glanis* (Matures) | 0.003 | 0 |
| *Silurus glanis* (Matures)—*Alburnus alburnus* | 0.220 | 0.003 |
| *Silurus glanis* (Matures)—*Pseudorasbora parva* | 0.162 | 0.001 |
| *Silurus glanis* (Matures)—*Lepomis gibbosus* | 0.217 | 0.227 |

SEAc, corrected Standard Ellipse Area considering 40% of core data.
SEAb, Bayesian Standard Ellipse Area, considering 95% of data.

## DISCUSSION

Multiple invaded communities are particularly interesting as most studies focus on the effects of a single introduced species (but see Preston et al., 2012; Jackson, 2015). In the Mediterranean area, to the best of our knowledge, only few studies explored the trophic interactions in highly invaded inland water communities despite the high frequency of species invasions (Galil, 2007; Di Castri et al., 2012). Furthermore, they both concern lentic waters and comprise generally fewer species. Like our study, combining dietary analyses and stable isotopes, Stellati et al. (accepted) described the trophic interactions of a pond community in Monterotondo (Rome, Central Italy), composed by almost exclusively invasive species except for top predators (*Natrix natrix* and *Natrix tesselata*). Using an identical approach, Haubrock et al. (2019a) investigated the relationships in Northern Spain in Arreo Lake dominated by invasive species. As shown by the studies above, such integrative approaches applied to a peculiar community can provide not only insights into trophic interactions among invasive species

but can also improve our ability to understand their synergistic impacts onto communities. However, in the study presented here, investigations were conducted during spring season in a completely different ecosystem: a highly anthropogenically altered river with no seasonal drought and no native fish predators. While the dataset cannot be seen as enough to make a generalization on the entire year, we believe that the collected data are sufficient to analyse the interactions and subsequent interactions among alien invasive species in the Arno river during spring season.

## Community Structure

The reconstructed community had a typical pyramidal shape, with the apex position being held by two invasive predatory fish, *I. punctatus* and juvenile *S. glanis*. Compared with the works of Stellati et al. (accepted) and Bissattini and Vignoli (2017), in which the community was composed—according to isotopic data—by approximately two trophic levels, in the Arno River it spanned over at least three trophic levels, indicating a higher complexity. However, it is interesting to note that this complexity is reached with a high number of species, while in Arreo Lake the same number of trophic levels was obtained with only four species, that had clearly separated niches, with a primary role of N isotopes signatures (and therein the trophic level) in this partitioning (Haubrock et al., 2019a). On the contrary, the Arno River community showed some overlap among trophic niches of fish species, but not a clear segregation of all niches as observed in Arreo Lake (Haubrock et al., 2019a), as the latter was the result of the low species number and a common geographic origin.

Estimations of trophic positions showed a strong top predator behavior for *S. glanis*, *I. punctatus*, especially in the juvenile stage, and also *L. gibbosus*, coherently with their known biology and other isotope studies (Werner et al., 1977; Vander Zanden and Rasmussen, 1999; Vander Zanden et al., 1999; Syväranta et al., 2010). Compared with Haubrock et al. (2019a), *L. gibbosus* was identified as a possibly secondary -not a primary- predator. This might be associated to the differences in ecosystem and community complexity. The functional category of primary predatory fish was represented by *P. parva*, *A. alburnus*, and *Padogobius* sp., although for the latter this position should be taken with caution due to the low number of sampled individuals (n = 4). *Cyprinus carpio* (n = 4) and the only specimen of *B. barbus* can be considered true omnivores as they had a TP intermediate between that of primary predators and herbivores. Commonly, *A. alburnus* and *P. parva* are known omnivores with a preference for zoobenthos (Politou et al., 1993; Gozlan et al., 2010). Nonetheless, they have the ability to exert wider, on higher trophic level focused feeding preferences (Vøllestad, 1985; Lammens and Hoogenboezem, 1991; Nagata et al., 2005), potentially explaining their comparatively elevated TPs (Syväranta et al., 2010; Jackson and Britton, 2013). However, the low number of individuals captured cannot allow other speculations, but rather give a rough indication of the positioning of these species trophic niche in the studied ecosystem. Among invertebrates, the invasive red swamp crayfish *P. clarkii* and the native *P. antennarius* were also included in this category. Compared with Arreo lake (Haubrock et al., 2019a), *P. clarkii* belonged to a higher trophic level, potentially due to the
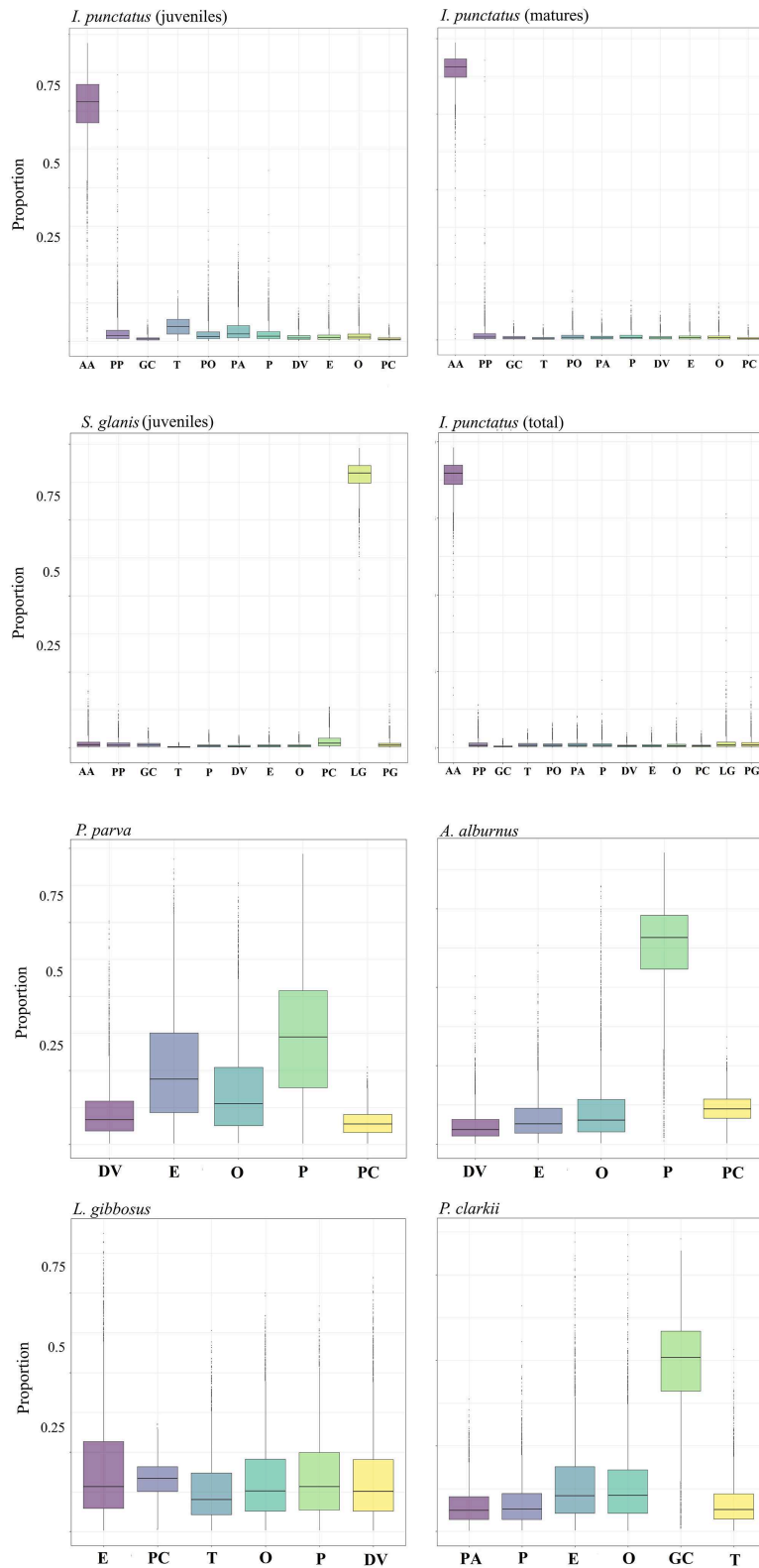
**FIGURE 2 |** Estimated proportions of prey items to the diet of: *Ictalurus punctatus* (juveniles), *Ictalurus punctatus* (matures), *Ictalurus punctatus* (total), juvenile *Silurus glanis*, *Pseudorasbora parva*, *Alburnus alburnus*, *Lepomis gibbosus*, and *Procambarus clarkii*. Prey items were considered those belonging to lower trophic levels without consideration of any prior. The analysis was performed using the R-package "simmr".
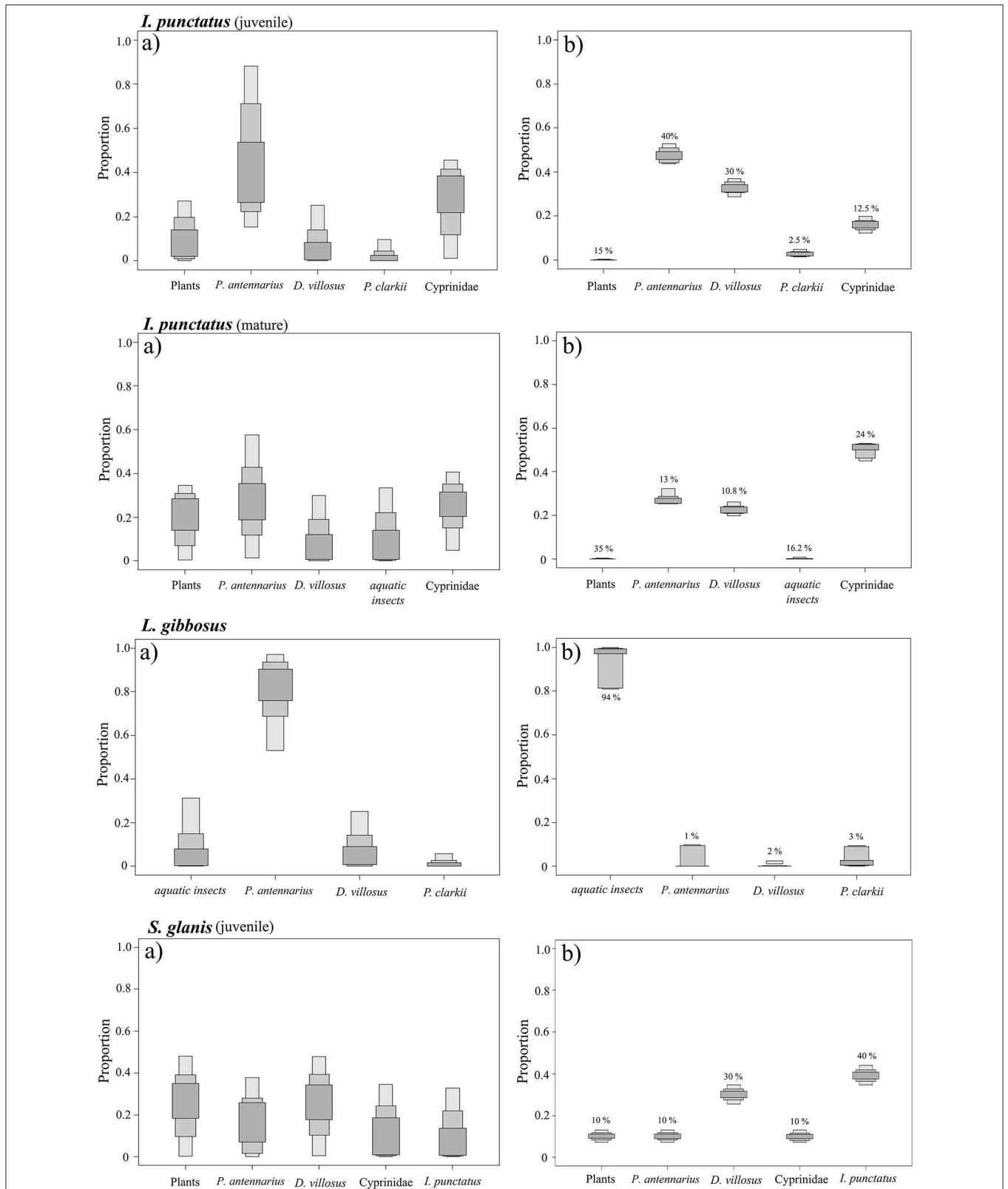
**FIGURE 3 |** Estimated contribution of consumed prey items to the diet of: *Ictalurus punctatus* (juveniles), *Ictalurus punctatus* (matures), *Lepomis gibbosus* and juvenile *Silurus glanis* using dietary priors. **(a)** Based on the 5* most consumed prey items; **(b)** including the number of occurrences (N%) of the 5* most consumed prey items (indicated in %). *: 4 in the case of *L. gibbosus*. The prior based analysis was performed using the R-package "SIAR" with credibility intervals of 95%.

| Source | df | SS | MS | Pseudo-F | P(perm) | perms | P(MC) |
|--------|-----|--------|--------|----------|---------|-------|-------|
| sp | 14 | 3293.1 | 235.22 | 135.34 | 0.001 | 999 | 0.001 |
| Res | 202 | 351.07 | 1.738 | | | | |
| Total | 216 | 3644.1 | | | | | |

greater complexity of the Arno River community and therefore higher number of potential prey items and advanced stage of eutrophication. As discussed for *B. barbus*, the trophic position of the omnivorous *T. tinca*, cannot be considered precise; however, both these samples are still indicative also for future studies on these two rare species in this or other ecosystems. Surprisingly, the larvae of predatory insects (Ephemeroptera and Odonata) as well as the invasive amphipod *D. villosus*, which is known to be a predator of other aquatic macroinvertebrates and fish eggs (Dick and Platvoet, 2000), fell in the same trophic level of primary consumers. This is maybe due to predation on unsampled invertebrate consumers, with a lower N isotopes signature than mollusks, leading to potential underestimations in TPs. Moreover, due to the generally high angling activity (using commonly float and feeder fishing) in the Arno river, an unpredictable influx of unnatural food sources cannot be excluded. These items could likely affect the trophic positioning of species like *T. tinca*. However, the angling activity in the Arno is considerably low during spring (pers. comm. Thomas Bussatto). Additionally, if such baited fishing would occur, used bait-items would have been observed within the diet of *I. punctatus* (Haubrock et al., 2018b, 2019b). Interestingly, juvenile *I. punctatus* had a slightly higher nitrogen signature than matures, which is uncommon for catfish species as they generally express higher N values with increasing total length (Syväranta et al., 2010). Considering the high level of eutrophication and the natural accumulation of nutrients (i.e., dead matter) in the river bottom, this observation can potentially be explained by the greater consumption of enriched detritus by juveniles (Hendrix et al., 1986; Hansen and Kristensen, 1998). Another explanation could be that adult *I. punctatus* lower their trophic position to avoid (or is confined to a lower position as a result of) high competition with other piscivorous fish like *S. glanis* (Fry, 1999) as they share similar niches. Because *S. glanis* is not a detrivorous species, an effect of N-enriched detritus can be neglected in this species' case. Nonetheless, it should be noted that the inclusion of adult specimens of *S. glanis* might lead to a significant correlation between TL and N signatures (Syväranta et al., 2010).

Moreover, several species expressed a negative correlation between TL and C signatures, suggesting consumption of prey items that feed on different aquatic or terrestrial vegetation. This underlines the scenario that these species change habits as they grow, with e.g., juveniles of *I. punctatus* living closer to the riverbank and relying on fallen terrestrial items more than matures, the latter feeding offshore. However, although Syväranta

et al. (2010) argued that in *S. glanis* $\delta^{15}$N increases with TL due to the ability to consume higher trophic prey, such an increase was only observed in *L. gibbosus*, indicating an ontogenetic size-based prey shift.

## Layman Metrics and Niche Overlap

Among fish species, Layman's metrics (Layman et al., 2007) showed a great variability in N signatures, thus indicating the utilization of prey items from different trophic levels in *P. parva*, *L. gibbosus*, and adults of *I. punctatus*, while juvenile *S. glanis* were quite specialized in its trophic role as apex predator. Carbon variability was largest in *A. alburnus*, suggesting the direct or indirect consumption of prey from different origins. Niche width measures showed the widest niche for *L. gibbosus*, *P. parva*, and juveniles of *I. punctatus*, thus, these species express the greatest intra-specific variability. In contrast, a more specialized niche was found for *S. glanis* together with a niche partitioning among catfish species with the potential to compete due to the generalist diet of *I. punctatus* (see Haubrock et al., 2019b for a detailed discussion). Moreover, the comparison between *A. alburnus* and *P. parva*, two ecologically similar species belonging to the same trophic level and with a considerable niche overlap, showed a considerably wider niche width for *P. parva* based on a considerable variability in $\delta^{15}$N values, probably due to a higher feeding variability and adaptability to many ecosystems, a key factor in its invasion history. Indeed, Rosecchi et al. (1993), Gozlan et al. (2010) and Yalçin-Özdilek et al. (2013) stated that *P. parva* is prone to predate, among other items, fish larvae as well as eggs. However, while these potential preys were present during the time of the sampling, they were not observed in the diets, as previously observed (Annamaria Nocita, unpubl. data). In contrast, *A. alburnus* showed significantly less inter-individual variability linked to feeding activity mostly relying on macroinvertebrates (Politou et al., 1993).

Similarly, it is interesting to compare the two size-matched crustaceans *D. villosus* and *P. antennarius*. The greater variability in $\delta^{15}$N was found in the former, suggesting the consumption of prey items from different trophic levels. However, *D. villosus* unexpected low trophic position raises questions on its specialized predatory behavior, whilst *P. antennarius*, despite being an omnivore feeding also on detritus and algae, occupied a higher trophic position with low variability in $\delta^{15}$N. Moreover, as for *P. parva*, *D. villosus* had a considerably wider niche than that of *P. antennarius*, a factor potentially related to the success of this invasive species. Finally, *P. clarkii* showed a wider niche compared with the other two crustaceans, particularly due to its variability in carbon sources. This was not surprising, giving the opportunistic and generalist diet, especially in invaded areas (Souty-Grosset et al., 2016).

Permutational analysis of variance of species niches identified considerable overlaps mainly between predatory fish species, confirming SEAc and SEAb overlap estimations. The result is a community structure more packed at higher than at lower trophic levels (**Figure 1**). Therefore, we speculate that, in the Arno River community, a diminished predator pressure could be not caused by the presence of a keystone species (such as in Bissattini et al., 2018), but by the potential competition among

predators and potentially occurring competition- avoidance mechanisms (Haubrock et al., 2018b).

## Mixing Models and Resource Utilization

Standard mixing models under the assumption of predation on lower trophic positions (**Figure 2**) presented limited, but representative, information, as this theoretical predation scenario is estimated based on the potential availability of prey items and the possibility of their consumption (Phillips, 2001; Phillips et al., 2005). However, including the observed prey and their occurrences in the diet in these models, considerable differences emerged (**Figure 3**). When using DA based priors, *I. punctatus* presented more precise proportions of prey contributions, while the diet of *L. gibbosus* is based more on aquatic insects, which is directly mirrored by DA results (**Table 1**). For *P. parva* and *A. alburnus*, where no suitable DA were applicable due to the low number of stomach contents, mixing models without priors showed a higher predation of *P. antennarius* by *A. alburnus*, but a wider more generalistic feeding by *P. parva*. In addition, using the five most commonly present prey items in the diet of *S. glanis* led to a considerable role of plants, which did not majorly contribute to its TP. Nonetheless, it has to be stated that only 19 of 39 *S. glanis* had full stomachs. In this regard, the presence of plants in the diet of *S. glanis* is particularly interesting, as firstly, *S. glanis* is only feed on plant matter during early invasion (Carol et al., 2009), thus life stages, and secondly, because symbiotic bacteria are needed for fish to be able to digest plants (Vejříková et al., 2016). However, accidental digestion of plant matter while preying on living prey may occur, the potential competition with *I. punctatus* cannot be excluded of having caused diet shifts within *S. glanis* immature life stage due to this species known generalism and adaptability to new prey sources (Vejřík et al., 2017b; Haubrock et al., 2019b).

Concluding, this latter prior-based method can be considered more accurate, due to the combination of two different time mediated methods (Moore and Semmens, 2008). Nevertheless, when information on the local diet are not available, the first method without prior can be considered as very informative (Pacioglu et al., 2019).

## A Case Study on Alien Species Communities?

The study river is a peculiar ecosystem, as the majority of the inhabiting species are alien. Moreover, the communities' hierarchical order indicates a typically intact community and highlights the foregone interactions that have led to this structure (McQueen et al., 1989; Polis, 1994). The main predatory species were observed to mostly feed on other alien species, and thus, resulting in complex and manifold species interactions. These differing interactions underline the need for studies on a case by case basis. Interestingly, combining SIA and DA revealed differing interactions according to whether alien species shared a common life-history or not.

In the case of *I. punctatus* and *L. gibbosus*, as these two species co-occur in the same ecosystems in North America (Werner et al., 1977; Smith, 1981) and thus, share a common life-history and overlapping native range as well as history of

co-evolution, some niche overlap was identified. Moreover, the applied PERMANOVA did not indicate a statistically significant difference between juvenile catfish and pumpkinseed trophic niches. The diet of both included a considerable percentage of aquatic insects, but the mixing models with and without prior showed a different contribution of prey items. More importantly, *I. punctatus* did not show any indication of predation on or direct competition with *L. gibbosus*. Hence, competition could be not direct, but rather based on the opportunistic nature of *I. punctatus* or other factors that lead to a slightly lower trophic position in mature than juvenile *I. punctatus*. Moreover, these species likely avoid competition with their different feeding activities (benthivorous generalist vs. pelagic opportunist). On the other hand, where potentially competing alien species came from different geographical ranges, observations were slightly different. *Pseudorasbora parva* and *A. alburnus*, two species with similar behavior and activity pattern, showed major isotopic niche overlap confirmed also by the PERMANOVA *post-hoc* test, suggesting that, if resources become limiting, these species are likely to compete.

As for the two apex predators, the results indicated that while both, mature and juvenile *I. punctatus* show a medium to high diet overlap with *S. glanis*, a minor isotope overlap was only observed between juvenile *I. punctatus* and *S. glanis* but not among life-stages of *I. punctatus* (Haubrock et al., 2019b). It has therefore to be considered that *I. punctatus* is a primarily benthic forager, a factor that likely limits the competition with other species. These results led to the conclusion that both life-stages of *I. punctatus* show a potential to compete with juvenile *S. glanis* but not with each other, likely due to diet- and behavioral-based intra-specific avoidance mechanisms. However, it should be noted that including mature individuals of *S. glanis*, as shown in Syväranta et al. (2010) and Vejřík et al. (2019), might have an effect of this species' niche occupation and the observed niche partitioning (Haubrock et al., 2019b). With the use of mixing models, different preferred prey items were identified for both species, while the use of priors, when applicable, indicated a considerable feeding activity by *S. glanis* on *I. punctatus*.

Finally, the presence of *P. clarkii*, often described as a keystone species (Liu et al., 2018), was shown to lower the impact of *L. catesbeianus* on native amphibians due to *P. clarkii*'s availability as prey (Bissattini and Vignoli, 2017). However, in the Arno River it occurred at low densities. For this reason, the more abundant *P. antennarius* and *D. villosus*, both highly present in the diet of multiple species as well as indicated by the mixing models, probably reside in a similar function within the community. Therefore, in our study site, the presence of *P. clarkii* seemed not to lower impacts on present species by predatory fish, but rather contributed to it.

Not all alien species that originate from roughly the same geographic region share a common history of co-evolution. However, in this studies case, North American species indeed share the same ecosystems. As such, it was shown that not only alien species do differ in their ecological niche occupation, but the existence and type of interactions may differ according to the involved species and their respective histories of co-evolution. This is true despite the close

clustering of fish species within the 4th and 3rd trophic level. Indeed, our combined SIA and DA results suggest that, when two alien species originate from the same geographical range, competition seemed to be possible due to some diet overlap, but was neglectable due to varying feeding preferences, occupied niche space and potential avoidance mechanisms (Haubrock et al., 2018b), as indicated by prior (DA) - based mixing models and SEA$_{b/c}$. Nonetheless, when species originated from different geographic ranges but occupied the same trophic level, i.e., similar trophic positions, shared niche space and isotope overlap were more likely to occur. Resultant, due to the variability of occurring interactions, niche shifts or increasing competition could lead to an increased impact on native species, while predetermined interactions among species with common life-history could lead to a lowered feeding pressure. However, more studies are therefore needed in this direction to confirm these findings and to increase information on these alien species' impacts. These future sampling campaigns should also consider summer and autumn because behaviors and thus, interactions may differ (Vejřík et al., 2017a), potentially further enhancing our comprehension of mechanisms underlying biological invasions and species interactions.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## ETHICS STATEMENT

Ethical review and approval was not required for the animal study because this study was carried out in accordance with the principles of and under the restriction of the laws currently effective in Tuscany, Italy, following the recommendations of Dr. Annamaria Nocita. The protocol was approved by the local governmental authorities.

## AUTHOR CONTRIBUTIONS

PH, PB, and ET designed the study. PH, PB, MA, and WG performed research. PH, MA, and AI analyzed data. LV contributed new models. PH and PB wrote the first draft. All authors contributed to the paper and approved it.

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The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00355/full#supplementary-material

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