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Trophic structure of a pond community dominated by an invasive alien species: Insights from stomach content and stable isotope analyses

Alessandra Maria Bissattini¹  | Phillip J. Haubrock^{2,3,4} | Vincenzo Buono¹ | Paride Balzani⁵ | Nicolò Borgianni¹ | Luca Stellati¹ | Alberto Francesco Inghilesi^{4,5} | Lorenzo Tancioni⁶ | Marco Martinoli⁶ | Elena Tricarico⁵ | Leonardo Vignoli¹ 

¹Department of Biology, University of Roma Tre, Rome

²Department of River Ecology and Conservation, Senckenberg Research Institute and Natural History Museum Frankfurt, Gelnhausen, Germany

³Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, University of South Bohemia in České Budějovice, Vodňany, Czech Republic

⁴NEMO Ambiente s.r.l., Florence, Italy

⁵Department of Biology, University of Florence, Fiorentino, Italy

⁶Department of Biology, University of Rome 'Tor Vergata', Rome, Italy

Correspondence

Alessandra Bissattini, Department of Biology, University of Roma Tre, viale Marconi 446, 00146, Rome, Italy.
Email: alessandramaria.bissattini@uniroma3.it

Abstract

1. Invaders affect native species across multiple trophic levels, influencing the structure and stability of freshwater communities. Based on the 'trophic position hypothesis', invaders at the top of the food web are more harmful to native species via direct and indirect effects than trophically analogous native predators are.
2. However, introduced and native predators can coexist, especially when non-native species have no ecological and behavioural similarities with natives, occupy an empty niche, or natives show generalist anti-predator strategies that are effective at the community level.
3. At present, conservation efforts are focused on eradicating invaders; however, their removal may lead to unwanted and unexpected outcomes, especially when invaders are well established and strongly interspersed with natives. This highlights the need to consider invaders in a whole-ecosystem context and to consider the evolutionary history and behavioural ecology of natives and invaders before active management is applied.
4. Here, stomach content and stable isotope analyses were combined to investigate a pond system dominated by invaders in order to understand the effects of the interactions among upper level predators and lower level members of the food web on the whole community structure.
5. Both diet and isotope analyses showed that several invaders contributed to the diet of natives and invaders. A significant isotope overlap was found among upper level predators. However, stomach content analysis suggested that predators reduced the potential competition differentiating the food spectrum by including additional prey in their diet. Both native and non-native upper level predators, by

Alessandra Maria Bissattini and Phillip J. Haubrock contributed equally.

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preying on invaders, seem not to exert a strong suppressive effect through predation and competition on native species.

6. This research confirms the importance of studying food webs to identify ecological conditions that forecast the potential for deleterious impacts before management is applied. In cases where invaders cannot be eradicated, management efforts should follow a conciliatory approach promoting the coexistence of native species with invaders.

KEYWORDS

conciliatory approach, feeding ecology, food web, interactions, predators, species facilitation, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$

1 | INTRODUCTION

A growing proportion of ecosystems worldwide host novel assemblages of native and introduced species (Wallach, Ripple & Carroll, 2015). This is particularly true in freshwater ecosystems, which are vulnerable to biological invasions owing to the strong link of humans to water, the inconspicuousness of aquatic ecosystems (Rico-Sánchez et al., 2020), and the high interconnectedness, and thus the dispersal ability, of freshwater species compared with terrestrial species (Selge, Fischer & van der Wal, 2011; Tricarico, Junqueira & Dudgeon, 2016). As a result, freshwater communities are often dominated by multiple invasive alien species (IAS) living in sympatry and interacting with each other (Gamradt & Kats, 1996; Kiesecker & Blaustein, 1998; Haubrock et al., 2020), and some of these may have facilitative effects (Crane et al., 2020).

Invasive alien species are known to affect the distribution and abundance of native organisms across multiple trophic levels, influencing the structure and stability of invaded freshwater communities (Strayer, 2010; Strayer et al., 2019). Based on the 'trophic position hypothesis', the magnitude of observed changes is strongly related to the position of IAS within the food web (Thomsen et al., 2014; Gallardo et al., 2016). This suggests that alien predators, invading the top of the food web, are more harmful than trophically analogous native predators on native biota belonging to lower trophic levels via direct effects (i.e. predation and competition) and indirect effects (i.e. habitat and behavioural alterations), owing to the naiveté of native species (Salo et al., 2007; D'Amore, Kirby & McNicholas, 2009). Even native predators, however, can be vulnerable to invader impacts (Dorcas et al., 2012), and they are amongst the most threatened groups globally (Dalerum et al., 2009), with cascading consequences on lower trophic levels ('trophic downgrading'; Estes et al., 2011). In particular, the suppressive, top-down control exerted by native predators on lower trophic levels could be compromised, resulting in deleterious smaller predator invasions and outbreaks ('mesopredator release hypothesis'; Zavaleta, Hobbs & Mooney, 2001; Wallach, Ripple & Carroll, 2015).

Although IAS can contribute to native predator extinctions (Dorcas et al., 2012), native and non-native predators can stably coexist (Letnic et al., 2009; Wallach, Ripple & Carroll, 2015). This usually happens

when invaders have no ecological and behavioural similarities with natives and occupy an otherwise empty niche (Adams & Pearl, 2007; Goodenough, 2010), or natives show generalist anti-predator strategies that are effective at the community level (Cox & Lima, 2006). Once multiple non-native species become established, their combined impacts can be additive or non-additive (Jackson et al., 2017). However, most often, the combined effects of non-native predators on native prey species are lower than the expected sum of individual effects owing to antagonistic interactions among IAS (Jackson, 2015; Wasserman et al., 2016; Bissattini & Vignoli, 2017; Jackson et al., 2017; Bissattini, Buono & Vignoli, 2018; Bissattini, Buono & Vignoli, 2019). Indeed, there are several cases in which non-native prey represents an alternative food source for non-native predators, decreasing the predation pressure on native resources (Karl & Best, 1982; Murphy & Bradfield, 1992; Liu et al., 2015; Bissattini, Buono & Vignoli, 2018; Liu et al., 2018; Bissattini, Buono & Vignoli, 2019). This suggests that invaders in different trophic positions may replace the ecological role of extinct taxa, mitigating the trophic downgrading (Cucherousset, Blanchet & Olden, 2012).

At present, conservation efforts are focused on eradicating invaders, but those that are well established and strongly interspersed with natives cannot realistically be eradicated without affecting native species (i.e. with the use of rotenone; Bellingan et al., 2019). Accordingly, IAS removal may lead to unwanted and unexpected outcomes (Zavaleta, Hobbs & Mooney, 2001; Wallach, Ripple & Carroll, 2015). Thus, freshwater communities dominated by IAS that appear to call for eradication may instead demand a conciliatory approach, managing the coexistence of natives with invaders in the long term (Schlaepfer et al., 2005; Carroll, 2011). Upper level predators, in particular, could be used to manipulate ecological processes and species abundances to achieve biodiversity conservation goals on the basis of their key-stone role in community structure and function (Beyer et al., 2007; Ripple & Beschta, 2007). However, although researchers have begun to explore the implications of multiple, interacting invaders, little attention has been paid to the implications of these interactions for eradication efforts (Zavaleta, Hobbs & Mooney, 2001). This highlights the consequential need to consider IAS in a whole-ecosystem context and to consider carefully the evolutionary history and behavioural

ecology of natives and invaders before active management is applied (Carroll, 2011).

Here, a system of ponds dominated by IAS was analysed by combining two different approaches: stomach content analysis and stable isotope analysis. Stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope ratios are a useful tool to describe qualitatively and quantitatively how organisms interact via diet, highlighting the potential trophic overlap and competition among species (Haubrock et al., 2018a; Bissattini, Buono & Vignoli, 2019). Stable isotope analysis can be used to compare the trophic ecology and the effect of IAS on trophic levels, thus estimating their impact on local communities (Balzani et al., 2016; Haubrock et al., 2019a; Haubrock et al., 2019b). In contrast to stomach content analysis, which provides a snapshot of the recently ingested items, stable isotope analysis provides relatively long-term and time-mediated information on the effect of consumed resources on a consumer isotopic signature (Huckembeck et al., 2014). Stable isotope analysis is based on predictable changes in N and C isotope ratios between consumers and their food sources (Post, 2002): $\delta^{15}\text{N}$ indicates trophic position within a food web, whereas $\delta^{13}\text{C}$ identifies major energy sources.

The main goal of this study was to analyse the interactions among upper level predatory species (native and non-native) and lower level members of the food web. Accordingly, the effects of multiple invasions on the structure of the whole community was estimated. Hence, this study specifically aimed at (i) describing the trophic structure at the community level, (ii) investigating the composition and contribution of prey in the diet of the species, (iii) evaluating the ecological interactions among native and non-native taxa, and (iv) analysing the role and potential impact of invaders on the community structure and the persistence of native species. An integrative approach has been proposed in order to drive future novel management strategies in multiple-invaded communities where invaders, well established and strongly interspersed with natives, cannot or should not be eradicated.

2 | METHODS

2.1 | Study area

Fieldwork was carried out in Monterotondo Scalo, a wetland area (surface area: 9 ha; perimeter: 1 km) located 20 km from Rome (central Italy; $42^{\circ}03'52.91''\text{N}$; $12^{\circ}35'07.83''\text{E}$; 18 m a.s.l.). The study area comprises four semi-natural ponds (surface area: 0.03–2 ha; depth: 1–1.8 m) derived from an abandoned clay quarry filled with waste material and meteoric water. Study ponds are closely positioned (mean distance: 3 m) and interconnected through the Tiber River flood events, thus sharing similar biotic and abiotic characteristics. The vegetation mainly consists of *Phragmites australis* (Cav.) Trin. Ex Steud., *Typha latifolia* (Linnaeus), and hygrophilous trees of *Alnus glutinosa* (Linnaeus) Gaertner, *Populus alba* (Linnaeus, 1753), and *Salix alba* (Linnaeus, 1753).

The pond system hosts a few native species: the water snakes *Natrix natrix* (Linnaeus, 1758) and *Natrix tessellata* (Laurenti, 1768), as

well as the green frogs *Pelophylax esculentus* (Linnaeus, 1758) and *Pelophylax lessonae* (Camerano, 1882). The two green frog species were treated as a single species (*Pelophylax* skl. *esculentus*) as they are impossible to distinguish in the field, being morphologically almost identical (Vignoli, Luiselli & Bologna, 2007a; Vignoli, Luiselli & Bologna, 2007b). The remaining vertebrate fauna is composed of invaders: six Eurasian fish species (the goldfish *Carassius auratus* (Linnaeus, 1758), the carp *Cyprinus carpio* (Linnaeus, 1758), the chub *Squalius squalus* (Bonaparte, 1837), the bream *Abramis brama* (Linnaeus, 1758), the roach *Rutilus rutilus* (Linnaeus, 1758), and the stone moroko *Pseudorasbora parva* (Temminck & Schlegel, 1846)) and four North American species (the black bullhead catfish *Ameiurus melas* (Rafinesque, 1820), the pumpkinseed *Lepomis gibbosus* (Linnaeus, 1758), the American bullfrog *Lithobates catesbeianus* (Shaw, 1802), and the red swamp crayfish *Procambarus clarkii* (Girard, 1852)).

2.2 | Fieldwork and laboratory analysis

Sampling was performed during the summer of 2016 (June–July). Different collection methods were adopted: (i) electrofishing for fish; (ii) hand catching for snakes; (iii) angling for frogs; (iv) baited traps for crayfish; (v) dip-netting for aquatic insects (Gyrinidae and Dytiscidae), algae, and detritus; (vi) pitfall traps for terrestrial insects (Carabidae, Curculionidae and Gryllotalpidae) and Isopoda, Diplopoda, and Gastropoda. Basal organic resources (i.e. leaf litter materials) were not sampled extensively, as the focus was on predators (Vander Zanden & Rasmussen, 1999). The procedures used for fish sampling were carried out in agreement with relevant legislation (CEN EN 14011/2003: Water quality – Sampling of fish with electricity) and were authorized by the Direzione Regionale Agricoltura e Sviluppo Rurale, Caccia e Pesca of the Latium Region (Det. G03436).

To obtain stomach contents, crayfish and fish were dissected, whereas frogs were flushed (Solé et al., 2005) and subsequently marked with the toe-clipping method (Phillott et al., 2007). Water snakes were marked by clipping ventral scales (Winne et al., 2006) and recently ingested prey items were collected by forced regurgitation (Fitch, 1987). Snout-vent length of frogs, total length of fish and water snakes, and cephalothorax length of crayfish were measured (accuracy: 0.01 cm).

Muscle from fish and crayfish, scale clips from water snakes, and tissue from clipped frog toes were taken for the analyses, whereas invertebrates were processed as whole individuals. Samples from tissues were preserved at -20°C , freeze-dried at -60°C for 24–48 hr (FD-10 Freezing Dryer), and then homogenized into a fine powder using a mortar and pestle at the University of “Roma Tre” in Rome (Latium, Central Italy). From each sample, 0.25 mg of powder was weighed on a Mettler Toledo AG245 microscale, packed in 53.5 mm tin capsules, and combusted with an elemental analyser (FlashEA 1112) connected to an isotope ratio mass spectrometer (Thermo Finnigan Delta Plus Advantage) at the “Institute of Environmental Geology and Geoenvironment of the Italian National Research Council” in the province of Rome (Latium, Central Italy). Two replicates for

each sample were measured. C and N isotope compositions were expressed in standard δ notations ($\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ with units of per mille) according to the equation $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1,000$, where R is $^{13}\text{C}:^{12}\text{C}$ or $^{15}\text{N}:^{14}\text{N}$. Results were referred to Vienna Pee Dee Belemnite for C and to atmospheric N_2 for N. To describe the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for all samples, the arithmetic mean plus/minus one SD was used.

2.3 | Stomach content analysis

Prey composition of each stomach was estimated by counting the number and frequency of prey (Callegari-Jacques, 2006). Niche width was estimated using the formula

$$B_i = \frac{\frac{1}{\sum_{j=1}^n p_j^2} - 1}{n - 1}$$

where B_i is the standardized index of niche breadth, p_i is the proportion of food category in the diet of species i , and n is the number of food categories ingested by species i (Levins, 1968; Hurlbert, 1978). Niche width was considered as low (0–0.39), intermediate (0.4–0.6), or high (0.61–1) (Grossman, 1986). Niche overlap was estimated using the formula proposed by Pianka (1967). RA2 (amphibians) and RA3 (fish and snakes) randomization algorithms were used to test for non-random patterns of resource utilization (Albrecht & Gotelli, 2001; Luiselli, 2006; Vignoli & Luiselli, 2012; Vignoli, Bissattini & Luiselli, 2017). Null models were built using EcoSim software (Gotelli & Entsminger, 2011).

2.4 | Stable isotope analysis

Trophic positions were calculated using a two-baseline (algae and detritus) Bayesian approach (R package `TROPHICPOSITION`; Quezada-Romegialli et al., 2018) and including trophic discrimination factors (McCutchan et al., 2003). The six Layman's metrics were estimated to quantify intra-specific and community niche width and variability (R package `SIAR`; Parnell et al., 2010): (i) range of $\delta^{15}\text{N}$ (NR), representing the number of trophic levels; (ii) range of $\delta^{13}\text{C}$ (CR), estimating the niche diversification at the base of the food web; (iii) total convex hull area encompassing all individuals (TA), representing the total extent of trophic diversity within the food web; (iv) mean distance to the centroid (CD), indicating the average degree of trophic diversity within the food web; (v) mean nearest neighbour distance (NND), indicating the overall density of species clustering and packing; and (vi) SD of the nearest neighbour distance (SDNND), representing the evenness of species packing (Layman et al., 2007). *Abramis brama*, *C. carpio* and *S. squalus* were excluded from further analysis since only one specimen per species was collected.

Differences in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signatures among species were analysed using a permutational multivariate analysis of variance (PERMANOVA) followed by pairwise (post hoc) tests. A canonical analysis of principal coordinates CAP (Anderson, Gorley &

Clarke, 2008) was conducted to analyse the detected differences. Spearman correlations between CAP axis and the original set of data were calculated to determine how the two variables affected the differences among species. PERMANOVA and CAP analysis were performed with PRIMER v. 6 (Clarke, 1993).

Isotopic niche overlap among predators was investigated by estimating (i) the corrected standard ellipse area (SEAc; corresponding to 40% of core data; R package `SIBER`; Jackson et al., 2011) and (ii) the probability of a species appearing within the niche space of another species (R package `nicheROVER`; Lysy, Stasko & Swanson, 2014). The kernel utilization density method was used to describe the isotopic niches for all the predatory species using a non-parametric estimator that precisely captures the distribution of the data and yields an accurate delineation of niche space and overlap (R package `rKIN`; Eckrich et al., 2020). Graphical representation and ranges (min–max) were used to assess the potential for competition between predatory species (Eckrich et al., 2020).

Stable isotope mixing models were used to examine the contribution of food sources to the isotopic signatures of collected individuals. These were supplemented with stomach content data (percentage of prey occurrences) as priors and trophic discrimination factors (R package `simmr`; Parnell & Inger, 2016). The results are presented as the average percentages with their SD .

3 | RESULTS

3.1 | Trophic community structure

Overall, $\delta^{15}\text{N}$ ranged between 1.423‰ and 10.902‰, whereas $\delta^{13}\text{C}$ ranged between –28.060‰ and –20.375‰. Mean isotope values were higher in water snakes, followed by adult bullfrogs and green frogs, fish species and juvenile bullfrogs, and aquatic and terrestrial invertebrates (Table 1; Supporting Information Appendix S1). PERMANOVA returned significant differences in species $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ levels, especially among top predators, whereas fish species showed well-defined values of isotopic signatures, except for the species pairs *A. melas* - *L. gibbosus*, and *R. rutilus* - *P. parva*. *Procambarus clarkii* did not differ in isotope signatures from terrestrial and aquatic insects (Supporting Information Appendix S2). CAP analysis (CAP1 and CAP2 axis squared canonical correlations equal to 0.80 and 0.34, respectively) revealed that $\delta^{15}\text{N}$ was inversely correlated with CAP1 (CAP1: $\delta^{15}\text{N}$ $r_s = -0.99$; $\delta^{13}\text{C}$ $r_s = 0.20$) and $\delta^{13}\text{C}$ was positively correlated with CAP2 (CAP1: $\delta^{15}\text{N}$ $r_s = 0.05$; $\delta^{13}\text{C}$ $r_s = 0.98$; Supporting Information Appendix S3). Water snakes were similar in $\delta^{15}\text{N}$ but differed in $\delta^{13}\text{C}$. Adult bullfrogs were similar in $\delta^{15}\text{N}$ to *P. skl. esculentus* but showed higher $\delta^{13}\text{C}$ levels; bullfrog juveniles were lower for $\delta^{15}\text{N}$ than both conspecific adults and green frogs but similar to *P. skl. esculentus* for $\delta^{13}\text{C}$ levels (Supporting Information Appendix S3).

Overall, five trophic levels were identified. Trophic level 1 included algae and detritus as basal resources. Trophic level 2 consisted of diplopods, isopods, and aquatic insects, and trophic level 3 comprised crayfish, gastropods, terrestrial insects, planktivorous, and benthivorous fish

TABLE 1 Number (N) and mean percentage values (\pm SD) of nitrogen (^{15}N) and carbon (^{13}C) isotope ratios of consumers collected at the study site. Layman's metrics are shown: estimates of trophic position without (TP) and with (TP*) trophic discrimination factors, $\delta^{15}\text{N}$ range (NR), $\delta^{13}\text{C}$ range (CR), total area (TA), mean distance to the centroid (CD), mean nearest neighbour distance (NND), SD of the nearest neighbour distance (SDNND), and corrected standard ellipse area (SEAc)

Species	N	^{15}N (‰)	^{13}C (‰)	TP	TP*	NR	CR	TA	CD	NND	SDNND	SEAc
Nn	20	10.902 \pm 1.098	-26.026 \pm 1.999	4.471	4.886	4.284	8.508	18.658	1.895	0.752	0.668	6.571
Nt	11	10.385 \pm 1.075	-27.947 \pm 1.460	4.331	4.647	3.116	4.228	9.096	1.571	0.633	0.456	5.329
Lc a.	20	7.957 \pm 0.840	-24.394 \pm 0.662	3.582	3.796	2.520	2.734	5.014	0.949	0.367	0.323	1.750
Lg	20	7.526 \pm 0.433	-28.060 \pm 1.028	3.479	3.683	2.046	4.889	5.004	0.878	0.376	0.435	1.457
Pe	21	7.636 \pm 0.854	-23.242 \pm 1.364	3.479	3.708	3.829	5.244	12.287	1.288	0.541	0.514	3.844
Am	20	7.318 \pm 0.300	-27.499 \pm 1.318	3.406	3.711	1.144	4.292	2.288	1.076	0.260	0.174	1.054
Pp	20	6.661 \pm 0.607	-26.347 \pm 2.052	3.213	3.416	8.318	8.988	1.716	0.584	0.500	8.318	3.608
Lc j.	10	6.159 \pm 0.560	-22.838 \pm 0.754	3.051	3.217	1.457	2.148	2.426	0.812	0.410	0.251	1.471
Rr	3	5.577 \pm 0.147	-24.527 \pm 0.823	2.950	3.151	1.523	0.042	0.638	0.598	0.624	1.523	
Ti	22	5.157 \pm 0.444	-20.813 \pm 5.037	2.880	3.004	4.776	12.479	40.255	2.973	0.876	1.001	
Ca	20	5.438 \pm 0.717	-26.627 \pm 0.914	2.848	2.997	2.815	3.977	5.543	0.941	0.328	0.237	
Ga	20	4.516 \pm 1.927	-25.502 \pm 1.522	2.464	2.506	7.896	6.106	26.591	2.161	0.901	0.643	
Pc	21	4.168 \pm 0.515	-24.427 \pm 2.181	2.464	2.537	2.034	8.041	9.540	1.994	0.549	0.497	
Ai	13	4.197 \pm 0.323	-24.754 \pm 4.127	2.410	2.402	6.391	12.477	36.850	4.645	1.187	0.959	
Is	19	3.394 \pm 1.401	-25.693 \pm 0.889	2.241	2.284	5.806	3.148	12.495	1.414	0.653	0.673	
Di	3	1.423 \pm 0.227	-24.375 \pm 1.599	2.100	2.081							
Al	10	1.344 \pm 0.215	-7.145 \pm 0.380			0.663	1.363	0.456	0.349	0.215	0.167	
De	5	2.700 \pm 0.315	-17.920 \pm 0.482			0.600	0.920	0.552	0.446	0.543	0.037	
Co	278			na	na	14.446	25.582	208.496	3.769	0.315	0.323	

a.: adults; j.: juveniles; Nn: *Natrix natrix*; Nt: *Natrix tessellata*; Lc: *Lithobates catesbeianus*; Lg: *Lepomis gibbosus*; Pe: *Pelophylax skl. esculentus*; Am: *Ameiurus melas*; Pp: *Pseudorasbora parva*; Rr: *Rutilus rutilus*; Ti: terrestrial insects; Ca: *Carassius auratus*; Ga: *Gastropoda*; Ai: *Procambanus clarkii*; Is: *Isopoda*; Di: *Diplopoda*; Al: algae; De: detritus; Co: community; na: not applicable.

(*C. auratus* and *R. rutilus*), bullfrog juveniles, and *P. parva*. Trophic level 4 included intermediate fish (*A. melas* and *L. gibbosus*), *P. skl. esculentus*, and *L. catesbeianus* adults. Water snakes occupied the top predator position (trophic level 5; Table 1). Layman's metrics showed that the community spanned different trophic levels (NR = 208.5; Table 1). As for amphibians, green frogs showed higher TA, SEAc, and CR values than both adult and juvenile bullfrogs. Similar CD values were found, whereas NND and SDNND values revealed that the package degree between individuals (i.e. trophic redundancy) was lower in *P. skl. esculentus* (Table 1). In water snakes, all metrics were higher in *N. natrix* than in *N. tessellata* (Table 1). Among fish species, the greatest TA, SEAc, and CD were found for *P. parva*, whereas the lowest ones were for *R. rutilus*, although its metrics should be considered with caution given the low number of individuals caught (Table 1).

3.2 | Diet analysis: Stomach contents and isotope analysis

Water snakes fed on non-native fish (*A. melas* and *C. auratus*) with the addition of native frogs (*P. skl. esculentus*) for *N. natrix* and non-native crayfish for *N. tessellata*, as shown by both dietary and isotope data (Table 2; Figure 1). Both dietary and isotope data showed that adult bullfrogs relied heavily on *P. clarkii*, juveniles fed mostly on isopods, whereas isopods and terrestrial insects mainly contributed to the green frog diet (Table 2; Figure 2). Adult bullfrogs showed lower niche width values ($B_i = 0.137$) than both conspecific juveniles ($B_i = 0.682$) and green frogs ($B_i = 0.340$). Both dietary and isotope data revealed that plant material and aquatic insects were the most important food items for fish species, except for *A. melas* and *L. gibbosus* that fed also on crayfish and other fish (Table 2; Figure 3). Low values of niche width were more frequent, occurring in *A. melas* ($B_i = 0.394$), *C. auratus* ($B_i = 0.293$), and *L. gibbosus* ($B_i = 0.138$), with niche width values being intermediate for *P. parva* ($B_i = 0.482$) and high for *R. rutilus* ($B_i = 0.941$). Plant materials and detritus contributed the most to the diet of crayfish, resulting in intermediate niche width values ($B_i = 0.556$; Table 2; Figure 3).

Stomach content analysis revealed a significant diet overlap among predatory species, especially between juvenile bullfrogs and green frogs ($P_{\text{obs}} = 0.77$, $P_{\text{exp}} = 0.56$, $P_{\text{obs}} > P_{\text{exp}}$, $P = 0.04$) and adult bullfrogs and green frogs ($P_{\text{obs}} = 0.08$, $P_{\text{exp}} = 0.45$, $P_{\text{obs}} < P_{\text{exp}}$, $P < 0.001$). Niche overlap among fish species was significantly higher than the null model ($P_{\text{obs}} = 0.53$, $P_{\text{exp}} = 0.23$, $P_{\text{obs}} > P_{\text{exp}}$, $P = 0.002$). No significant diet overlap was detected between adult and juvenile bullfrogs ($P_{\text{obs}} = 0.39$, $P_{\text{exp}} = 0.51$, $P_{\text{obs}} < P_{\text{exp}}$, $P = 0.83$) and in water snakes ($P_{\text{obs}} = 0.40$, $P_{\text{exp}} = 0.50$, $P_{\text{obs}} > P_{\text{exp}}$, $P = 0.75$). Isotope analysis revealed that water snakes showed the highest overlap probability (51.2%), followed by *A. melas* and *L. gibbosus* (46.6%) and adult bullfrogs and green frogs (39.0%). Low overlap values were found among the remaining predators (<11%). The pairwise probability of niche overlap showed significant values among frogs, especially between *P. skl. esculentus* and both juvenile and adult *L. catesbeianus* (Table 3). Similarly, water snakes showed a high probability to occur in each other's niche (Table 3).

Among fish species, the highest overlap was found between *A. melas* and *L. gibbosus* (Table 3). The kernel utilization density method provided similar results. The estimated isotope niche overlap between water snakes (0.28–1.00) showed that the core area of *N. tessellata* was completely engulfed by the isotopic niche of *N. natrix*. However, water snakes expressed a medium overlap with each other (0.0–0.37). Both age classes of *L. catesbeianus* overlapped partially with *P. skl. esculentus* (juveniles: 0.08–0.54; adults: 0.12–0.85) but not with water snakes. Among fish species, *L. gibbosus* and *A. melas* showed the highest degree of overlap (0.15–0.97) (Supporting Information Appendix S4).

4 | DISCUSSION

4.1 | Main findings

This study demonstrates a propensity for IAS to become integral components of food webs, with a trophic level almost entirely represented by invaders in the study systems. Although all top predators were natives in these systems (*N. natrix* and *N. tessellata*), these biotas were highly dependent on invasive prey. Interestingly, Lake Naivasha in Kenya showed a similar food web structure dominated by invaders with up to three non-native species at each main trophic level except for top consumers, which remain exclusively composed of native species (Harper et al., 2002; Gherardi et al., 2011).

The introduction of a non-native predator (*L. catesbeianus*) at the second highest level of the study food web, linked by a predator–prey relationship to non-native *P. clarkii*, could have a marginal role in affecting the structure of the whole community, potentially because of the primacy of *L. catesbeianus*–*P. clarkii* interactions (Bissattini & Vignoli, 2017; Bissattini, Buono & Vignoli, 2018; Bissattini, Buono & Vignoli, 2019). In this context, unpredictable and unwanted outcomes following IAS removal in isolation are likely to occur since invaders are well established, strongly interspersed with native species, and connected at different trophic levels (Zavaleta, Hobbs & Mooney, 2001; Schlaepfer et al., 2005). Since native species are disproportionately represented at the top of the food chain, the timely recovering of snake populations is necessary to avoid potential secondary negative effects, such as the establishment or increase of other invaders, the loss of a trophic level, the elimination of further native species, and the replacement of their functional roles by non-native species (Zavaleta, Hobbs & Mooney, 2001). Indeed, water snakes were found at low densities and in poor condition, probably because of the consumption of unsuitable non-native prey (catfish and crayfish), suggesting that the presence of rich allodiversity may have adversely affected the top predator fitness (Stellati et al., 2019).

4.2 | Trophic community structure and diet analysis

The systems in this study revealed a compact structure with many species characterized by well-defined isotopic values, high trophic

TABLE 2 Number (N), mean size (SIZE), snout-vent length for frogs, total length for fish and snakes, and cephalothorax length for crayfish) and SD of consumers collected at the study site. Percentage and frequency of occurrence (in parentheses) of prey are shown

N	Nh	Nt	Lc a.	Lg	Pe	Am	Pp	Lc j.	Rr	Ca	Pc
	20	11	28	104	21	38	21	18	3	24	33
SIZE ± SD (cm)	71.78 ± 9.97	85.05 ± 18.89	19.15 ± 1.14	8.15 ± 1.32	6.15 ± 1.49	8.37 ± 1.90	6.08 ± 2.38	8.69 ± 2.75	15.73 ± 0.17	19.37 ± 1.90	6.64 ± 1.78
Coleoptera	0	0	6.82 (8.70)	1.59 (3.85)	12.25 (30.77)	8.09 (22.50)	0	15.39 (25.00)	0	0	13.16 (15.15)
Diptera	0	0	0	58.20 (39.42)	2.04 (7.69)	30.64 (42.50)	36.11 (25.00)	0	0	0	7.90 (9.09)
Diptera l.	0	0	0	0	0	0	0	2.56 (6.25)	0	0	0
Ephemeroptera	0	0	0	1.06 (1.92)	0	0	0	0	0	0	0
Hemiptera	0	0	0	0	4.08 (15.38)	0	0	0	0	0	0
Hymenoptera	0	0	4.55 (4.35)	0.53 (0.96)	20.41 (23.08)	0	0	5.13 (12.50)	0	0	0
Lepidoptera	0	0	2.27 (4.35)	0	4.08 (7.69)	0	0	2.56 (6.25)	0	0	0
Lepidoptera l.	0	0	0	0	2.04 (7.69)	0	0	10.26 (25.00)	0	0	0
Mecoptera	0	0	0	0	2.04 (7.69)	0	0	0	0	0	0
Orthoptera	0	0	0	0	0	0	0	5.13 (12.50)	0	0	0
Eggs unid.	0	0	0	2.12 (3.85)	0	1.73 (5.00)	2.78 (6.25)	0	0	0	0
Larvae unid.	0	0	0	2.65 (4.81)	0	0.58 (2.50)	0	0	0	0	2.63 (3.08)
Araneae	0	0	0	0	6.12 (23.08)	0	0	7.69 (18.75)	0	0	0
Pulmonata	0	0	6.82 (13.04)	0	2.04 (7.69)	0	0	15.39 (31.25)	0	0	0
Isopoda	0	0	2.27 (4.35)	0	44.9 (61.54)	0	0	23.08 (31.25)	0	0	0
Decapoda	0	25 (9.09)	70.46 (95.65)	2.12 (3.85)	0	2.89 (12.50)	0	10.26 (25.00)	0	0	0
Haptotaxida	0	0	2.27 (4.35)	0	0	0	0	0	0	0	0
Julidae	0	0	0	0	0	0	0	2.56 (6.25)	0	0	0
Anura a.	20 (20.00)	0	0	0	0	0	0	0	0	0	0
Fish a. unid.	0	0	0	2.12 (3.84)	0	0.58 (2.50)	2.78 (6.25)	0	0	0	0
Fish e. unid.	0	0	0	1.06 (0.96)	0	1.73 (5.00)	0	0	0	0	0
Cyprinidae	0	50 (18.18)	0	0	0	0	0	0	0	0	0
Ictaluridae	80 (5.00)	25 (9.09)	0	0	0	0	0	0	0	0	0
Birds	0	0	4.55 (8.70)	0	0	0	0	0	0	0	0
Algae	0	0	0	1.59 (2.88)	0	7.51 (30.00)	36.11 (81.25)	0	28.57 (66.67)	13.04 (12.50)	7.90 (12.12)
Plant material	0	0	0	3.70 (6.73)	0	9.83 (32.50)	16.67 (37.50)	0	42.86 (100.00)	8.70 (8.33)	21.05 (24.24)
Plant seeds	0	0	0	0	0	18.50 (2.50)	0	0	0	0	13.16 (9.09)
Detritus	0	0	0	23.28 (42.31)	0	17.92 (77.50)	5.56 (12.50)	0	28.57 (66.67)	86.96 (83.33)	34.21 (39.39)

a.: adult; e.: juvenile; l.: larvae; unid.: unidentified; Nn: *Natrix natrix*; Nt: *Natrix tessellata*; Lc: *Lithobates catesbeianus*; Lg: *Lepomis gibbosus*; Pe: *Pelophylax skl. esculentus*; Am: *Amelurus melas*; Pp: *Pseudorasbora parva*; Rr: *Rutilus rutilus*; Ca: *Carassius auratus*; Pc: *Procamburus clarkia*.

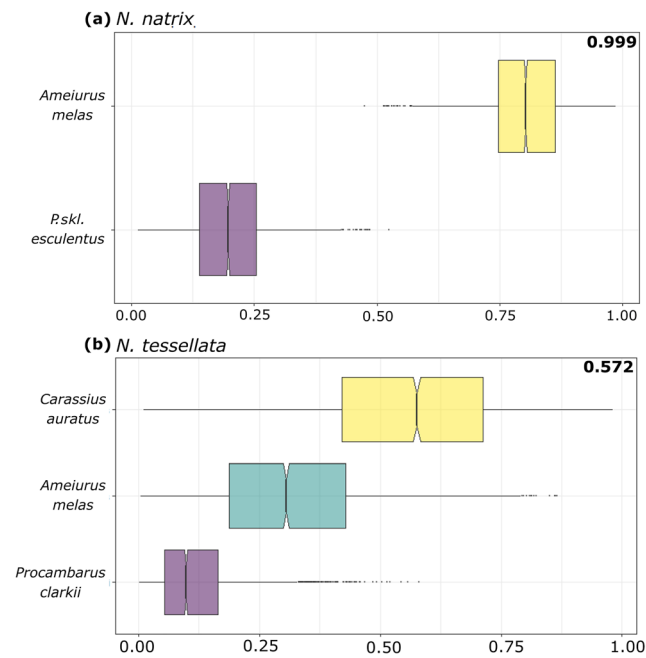


FIGURE 1 Results of stable isotope mixing models for (a) *Natrix natrix* and (b) *Natrix tessellata* based on diet priors and trophic discrimination factors. The number in the upper right corner shows the estimated probability of prey contributing to the predator trophic position

diversity, and low redundancy. Overall, five trophic levels were estimated; but an additional level could occur, which would include medium-sized mammals (i.e. foxes) and birds (i.e. herons) capable of preying on both snakes and frogs (Kupfer et al., 2006). Both isotope and dietary data showed that several invaders contributed to the diet of native and non-native species at multiple trophic levels. As expected, native water snakes were reported as top predators, because ephemeral freshwater wetlands often show simplified food webs dominated by reptiles owing to periodic dry periods (Gibbons et al., 2006; Stellati et al., 2019). Non-native fish contributed the most to the water snake trophic spectrum, with *N. natrix* also consuming native frogs in lower numbers. Generally, *N. tessellata* is specialized in feeding on fish, whereas *N. natrix* predominantly eats amphibians (Filippi et al., 1996); however, *N. natrix* is a very adaptable species, and it is known to feed on terrestrial and aquatic prey, especially when it occurs in syntopy with *N. tessellata* (Luiselli & Rugiero, 1991; Luiselli, Filippi & Capula, 2005). Surprisingly, juvenile bullfrogs were not consumed, which may originate from their relatively low availability in the study area (Metzger, Christe & Ursenbacher, 2011) or from *N. natrix* failing to adopt appropriate foraging skills to capture them (Cox & Lima, 2006).

Adult bullfrogs and green frogs were defined as intermediate predators because of their considerable role in energy flow and biomass conversion (Pough, 1980; Stewart & Woolbright, 1996).

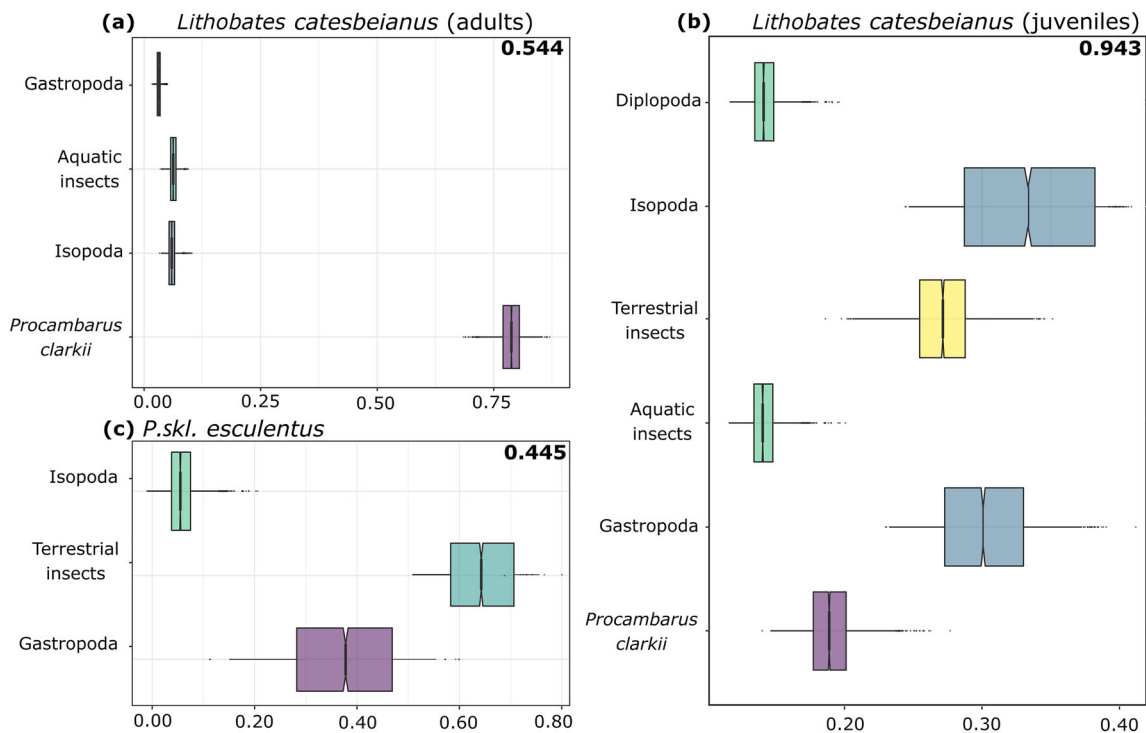


FIGURE 2 Results of stable isotope mixing models for (a) *Lithobates catesbeianus* adults, (b) *Lithobates catesbeianus* juveniles, (c) *Pelophylax sk. esculentus* based on diet priors and trophic discrimination factors. The number in the upper right corner shows the estimated probability of prey contributing to the predator trophic position

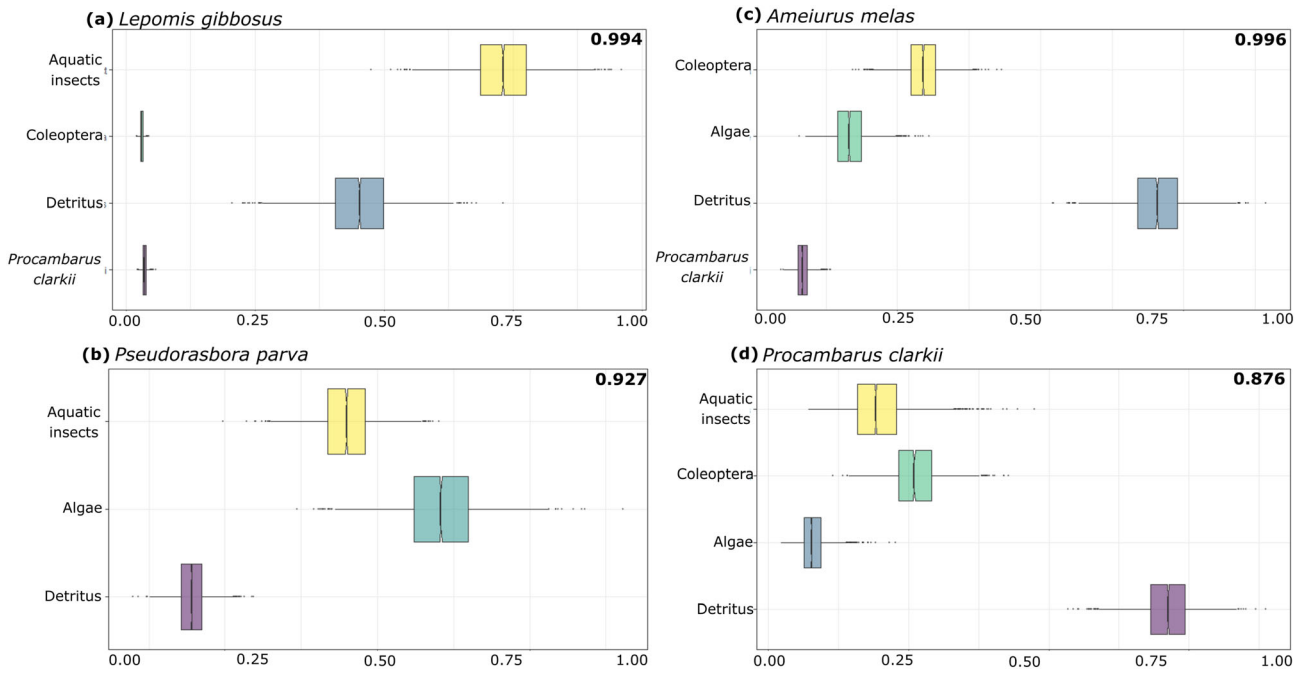


FIGURE 3 Results of stable isotope mixing models for (a) *Lepomis gibbosus*, (b) *Pseudorasbora parva*, (c) *Ameiurus melas*, and (d) *Procamburus clarkii* based on diet priors and trophic discrimination factors. The number in the upper right corner shows the estimated probability of prey contributing to the predator trophic position

TABLE 3 Posterior probability of overlap (i.e. the probability of a species A occurring within the niche of a species B) among predatory species at the study site

Species A	Species B									
		Am	Lc a.	Lc j.	Pe	Lg	Nn	Nt	Pp	Pc
Lc a.	2.5%	2	NA	1	29	0	0	0	10	0
	97.5%	25	NA	71	74	7	15	17	41	1
Lc j.	2.5%	0	1	NA	7	0	0	0	1	0
	97.5%	1	35	NA	50	0	0	0	25	20
Pe	2.5%	4	70	31	NA	0	0	0	19	0
	97.5%	59	99	99	NA	35	9	13	73	5
Lg	2.5%	62	0	0	0	NA	0	0	8	0
	97.5%	97	13	0	10	NA	5	12	50	0
Nn	2.5%	0	1	0	0	0	NA	55	0	0
	97.5%	29	50	0	19	61	NA	98	4	0
Nt	2.5%	0	0	0	0	0	34	NA	0	0
	97.5%	82	75	0	33	89	88	NA	34	0
Am	2.5%	NA	1	0	1	43	0	0	7	0
	97.5%	NA	37	1	20	84	2	5	40	0
Pp	2.5%	40	24	4	24	18	0	0	NA	0
	97.5%	100	82	86	85	90	2	7	NA	6
Pc	2.5%	0	0	0	0	0	0	0	0	NA
	97.5%	0	2	32	4	0	0	0	6	NA

a.: adult; j.: juvenile; Lc: *Lithobates catesbeianus*; Pe: *Pelophylax skl. esculentus*; Lg: *Lepomis gibbosus*; Nn: *Natrix natrix*; Nt: *Natrix tessellata*; Am: *Ameiurus melas*; Pp: *Pseudorasbora parva*; Pc: *Procamburus clarkii*; NA: not applicable.

Procambarus clarkii mostly contributed to the adult bullfrog diet, as previously found in other invaded areas (Liu et al., 2015; Bissattini & Vignoli, 2017; Bissattini, Buono & Vignoli, 2018; Liu et al., 2018; Bissattini, Buono & Vignoli, 2019). The strong preference of adult bullfrogs towards the red swamp crayfish probably results from their long coevolutionary history, both being native to North America and often coexisting inside and outside their historical geographical range (Bissattini, Buono & Vignoli, 2018). Terrestrial insects, Isopoda, Diplopoda, and Gastropoda mostly contributed to juvenile bullfrog and green frog diets (Bissattini, Buono & Vignoli, 2019). The present findings corroborate previous studies on the diet of juvenile *L. catesbeianus* (Willis, Moyle & Baskett, 1956; Govindarajulu, Price & Anholt, 2006), but partially disagree with those on *P. skl. esculentus*, feeding usually on both aquatic and terrestrial prey (Guidali, Scali & Carettoni, 2000; Vignoli, Luiselli & Bologna, 2009).

The diet spectra of fish mostly consisted of detritus, aquatic insects, and plant materials (Holopainen, Tonn & Paszkowski, 1997; Wolfram-Wais et al., 1999) with the addition of crayfish and small fish for *A. melas* and *L. gibbosus* (Leunda et al., 2008; Fobert et al., 2013). *Ameiurus melas*, *L. gibbosus*, and *P. parva* showed an invertivorous feeding habit, as already observed in the nearby Tiber River (Tancioni et al., 2001). Such species represent an essential intermediate link in the food chain by providing a continuum between higher and lower trophic levels, being both predators and prey (Berra, 2001). *Carassius auratus* and *R. rutilus* showed planktivorous and benthivorous habits by selecting food in sediments and ejecting all but the retained food particles back into the water column (Richardson, Whoriskey & Roy, 1995).

Aquatic and terrestrial insects played an important role in the food web as a main link between primary and secondary consumers (Pizzolotto, 1993), whereas the red swamp crayfish showed opportunistic feeding behaviour by consuming detritus, aquatic and terrestrial insects, and algae (Momot, 1995). Crayfish played a multifunctional role, acting as shredders and prey for other predators from several trophic levels (Momot, 1995).

4.3 | Species interactions

Overall, a significant isotope overlap was found among upper level predators; however, such an overlap does not necessarily imply competition, especially when resources are abundant (Giller, 1986). Moreover, as indicated by stomach content analysis, predators seemed to reduce the potential competition, expanding the food spectrum by including additional prey in their diet in order to limit the exploitation of shared resources (Vignoli, Bissattini & Luiselli, 2017).

No significant diet overlap was found between water snakes, probably because of the high number of empty stomachs observed; however, snakes are known to feed infrequently on large prey, characterized by both soft and hard tissues that are digested slowly (Cundall & Greene, 2000). By contrast, the isotope overlap was significant, probably because of the common consumption of *A. melas*. Diet overlap between the two species is generally low as there is a clear

partitioning of food resources (Luiselli & Rugiero, 1991; Filippi et al., 1996). Thus, it is likely that *N. natrix* and *N. tessellata* are forced to share most resources owing to the limited availability of native prey at the study site, and they mitigate potential competition by consuming different non-native fish, *A. melas* and *C. auratus* respectively (Luiselli & Rugiero, 1991).

Significant diet and isotope overlaps were found between juvenile bullfrogs and green frogs, although they occupy nearby but different trophic levels (Bissattini, Buono & Vignoli, 2019). This is probably a result of their similar sizes (Vignoli & Luiselli, 2012; Bissattini, Buono & Vignoli, 2019) or their similar microhabitat preferences (D'Amore, Kirby & McNicholas, 2009), being collected both on the pond banks and in shallow waters (Bissattini, Buono & Vignoli, 2019). Moreover, it has been observed that diet-tissue fractioning can vary among species and upon transition to adulthood (Martínez del Río et al., 2009; Trakimas et al., 2011), being affected by body size, growth rate, and protein turnover (Martínez del Río et al., 2009; Murray & Wolf, 2013).

A significant isotopic overlap was found between adult bullfrogs and green frogs, with the diet of the latter being nested in that of the former. This is likely to be due to the fact that amphibians are gape-limited predators (Vignoli, Bissattini & Luiselli, 2017), with the small-bodied green frogs having morphological restrictions on consumable prey (Schoener, 1974), whereas large-bodied bullfrogs consume the same food resources as *P. skl. esculentus* with the inclusion of larger prey (Stebbins & Cohen, 1995; Vignoli, Bissattini & Luiselli, 2017; Cuthbert et al., 2020).

Lithobates catesbeianus undergo ontogenetic habitat and dietary shifts (Bissattini, Buono & Vignoli, 2019): As adults, bullfrogs rely heavily on aquatic prey, whereas as juveniles they exploit several food resources, mainly of terrestrial origin (Díaz de Pascual & Guerrero, 2008; Bissattini, Buono & Vignoli, 2019). By increasing its trophic position during the life cycle, this species links terrestrial and aquatic food webs and affects prey communities in both habitat types (Whiles et al., 2006; Trakimas et al., 2011; Huckembeck et al., 2014; Bissattini, Buono & Vignoli, 2019). The isotopic overlap observed also suggests that such ontogenetic changes may lead to intra-specific facilitation by not competing for potentially common resources. In this context, it is likely that adult and juvenile bullfrogs shift their diet and habitat prevalence, thus introducing facilitative behaviour that favours rapid establishment of species and invasion success (Schoener, 1974).

The observed high degree of diet and isotope overlap among fish species could be related to their high trophic flexibility and the high availability of food resources in the study ponds (Godinho, Ferreira & Cortes, 1997; Leunda et al., 2008; Mohamed & Al-Jubouri, 2019). The isotope overlap found between *L. gibbosus* and *A. melas* probably resulted from their similar feeding behaviour: both species usually feed in benthic areas, consuming aquatic invertebrates, plant material, and other fish (Page & Burr, 1991; Declerck et al., 2002). Moreover, most of the *A. melas* collected were juveniles that were found to consume mainly aquatic insects and crustaceans (Page & Burr, 1991; Declerck et al., 2002). However, competition is unlikely to occur in the study area, as both *L. gibbosus* and *A. melas* usually demonstrate

substantial dietary opportunism by feeding on the most frequent and abundant prey (Godinho, Ferreira & Cortes, 1997; Leunda et al., 2008); selection of abundant prey, and avoidance of rare prey, could alleviate the ecological impacts of IAS and promote community stability (Cuthbert et al., 2018).

4.4 | Effects of species interactions on native amphibians

By preying on IAS, both native and non-native upper level consumers seem not to exert a strong suppressive effect through predation and competition on and with native species. This suggests that IAS occurring in keystone positions may not strongly influence the structure of the whole community when interacting with each other (Polis & Strong, 1996). Adult bullfrogs, by relying heavily on *P. clarkii* as their main food source, potentially reduce their predatory impact on native frogs that otherwise represent a preferred prey in the absence of crayfish (Liu et al., 2015; Bissattini, Buono & Vignoli, 2018; Liu et al., 2018; Bissattini, Buono & Vignoli, 2019). Similarly, the exploitation of non-native fish by *N.atrix* may also mitigate the predatory pressure on native amphibians that usually represent its elective prey. The present suggestions corroborate Polis & Strong (1996), who suggested that bottom-up and top-down effects in communities are compromised when allochthonous resources occur at both the basal and the top consumer levels. However, the competition impact of juvenile bullfrogs on green frogs cannot be excluded because of the high degree of diet overlap detected (Stewart & Sandison, 1972; Werner, Wellborn & McPeck, 1995; Bissattini, Buono & Vignoli, 2019). Indeed, resource-limited conditions, which are likely to occur when the study ponds periodically dry up, may increase the trophic niche overlap between juvenile bullfrogs and green frogs (Kuzmin, 1995; Bissattini, Buono & Vignoli, 2019), affecting their coexistence in the long term, resulting in green frog niche displacement, reduction in fitness, or even extinction at local scale (D'Amore, Kirby & McNicholas, 2009). Moreover, it is conceivable that the prey availability in the study area did not generally satisfy the trophic needs of both species of water snakes. Indeed, those individuals collected were smaller than those described from neighbouring localities, thus confirming the observations of Stellati et al. (2019) in the same study area. The same authors also found individuals in poor body condition and injured by catfish and crayfish spines. This suggests that the absence of prior reciprocal evolution among *Natrix* species and non-native prey may disadvantage native water snakes, leading to a reduction in energy acquisition, and ultimately affecting their survival (Wallach, Ripple & Carroll, 2015). The feeding activity of water snakes may be also disturbed by adult bullfrogs through predation, competition, and interference, as already reported in previous studies (Minton, 1949; Smith, 1977). Indeed, a case of predation of *N. tessellata* by *L. catesbeianus* has been found to occur in the study area (Bissattini, Buono & Vignoli, 2019; Stellati et al., 2019). Nevertheless, the long-term ecological and evolutionary feedbacks between IAS and the invaded communities are still unknown, as well as the

effects that the long-term interactions may have on community conservation.

4.5 | Conservation implications

This study confirms the importance of studying food webs and functional roles to identify the ecological conditions that forecast the potential for deleterious impacts before management is applied (Zavaleta, Hobbs & Mooney, 2001). In cases where invaders cannot or should not be eradicated, a conciliatory approach, managing the coexistence of native and non-native species, may represent a more successful alternative to eradication (Carroll, 2011; Bissattini, Buono & Vignoli, 2019). Indeed, species with high interconnectivity and a common co-evolutionary history are known to be more resilient to control and eradication than less connected species are (Haubrock et al., 2018b; Haubrock et al., 2019b). In this context, the recovery of native top predators (e.g. *Natrix* species) may provide better outcomes than lethal control of invaders (e.g. *L. catesbeianus*), whose removal may facilitate population outbreaks of other interconnected invaders (e.g. *P. clarkii*), with a cascade of damaging impacts on native species (e.g. *P. skl. esculentus*) (Wallach, Ripple & Carroll, 2015).

One strategy that might allow the coexistence of native and non-native species is to integrate behavioural and evolutionary processes into conservation and management plans ('evolutionarily enlightened management'; Ashley et al., 2003; Schlaepfer et al., 2005). The main aim of this proposed approach is to promote the survival of a native species long enough to allow a transition to its novel selective regime (Schlaepfer et al., 2005). Based on this premise, efforts should be focused primarily on the creation of conditions in which natives are subject to a selective and sustainable pressure that could drive an evolutionary change in behaviour without causing the extinction of local species (Schlaepfer et al., 2005). This approach could be particularly useful in systems where (i) the eradication of some invaders (e.g. *P. clarkii*) is unfeasible (Wizen et al., 2008) and (ii) the interplay among non-native species (e.g. *L. catesbeianus*-*P. clarkii*) produces both positive (e.g. *P. skl. esculentus*; Bissattini, Buono & Vignoli, 2018; Bissattini, Buono & Vignoli, 2019) and negative (*Natrix* species; Stellati et al., 2019) effects. It has also been suggested that 'evolutionarily enlightened management' is effective for species with small ranges, large populations, and sufficient genetic variability (Goodenough, 2010).

The identification of the environmental and biological conditions promoting the long-term survival of the species is crucial for the implementation of this approach (Laha & Mattingly, 2006). For instance, Adams & Pearl (2007) pointed out that native and non-native species are more likely to coexist when they differ in natural history and microhabitat preferences. This suggests that the maintenance or restoration of suitable habitats may support the recovery of native species and limit the distribution of invaders (Adams, 1999; Adams & Pearl, 2007). The increase in habitat diversity, through the creation of temporal and spatial refugia, may mediate the interactions between native and non-native species, eventually favouring the

emergence of traits that facilitate their long-term survival (Schlaepfer et al., 2005; Adams & Pearl, 2007). In the present study site, such an approach may help water snakes and green frogs to develop mechanisms (e.g. increased escape speed, increased predator detection ability) facing the direct predation, interference, and competition pressures exerted by bullfrogs and favouring the exploitation of new types of resources. In areas with a mix of refugium and non-refugium habitats, native species could evolve or learn mechanisms surrounding invasions, ultimately persisting on their own within a few generations (Phillips & Shine, 2004; Wallach, Ripple & Carroll, 2015). Native snakes *Pseudechis porphyriacus* and *Dendrelaphis punctulatus* showed a steady reduction in gape size and an increase in body length during the 50 years following the *Bufo marinus* invasion (Phillips & Shine, 2004). Moreover, *P. porphyriacus* behaviour and physiology evolved in response to toxic cane toads so rapidly (fewer than 23 generations) that snakes avoid *B. marinus* consumption and develop high bufotoxin resistance (Phillips & Shine, 2006). Similarly, *Rana aurora* exposed to *L. catesbeianus* for 70 years is able to detect its chemical cues and to avoid its predation (Kiesecker & Blaustein, 1997). It is therefore reasonable to assume that the evolution of traits facilitating the long-term survival of native species in coexistence with invaders could develop even more rapidly if assisted through a proper 'evolutionarily enlightened management'.

The management approach proposed here may be effective in multiple-invaded communities with a similar species composition and food web structure. This study, however, provided only a snapshot of the structure of the community from a trophic point of view, being unable to predict how the current situation will evolve in future, as (i) the species composition may shift towards those invulnerable to invaders, or other invaders might enter the assemblage, (ii) the effects of invaders may accumulate through time, or (iii) invaders may interact with others, producing effects that change over time (Strayer, 2010). Moreover, the impacts of invaders may vary widely, spatially and temporally, suggesting that non-native species should be considered in a whole-ecosystem context and in light of continuing invasion-influenced evolution (Carroll, 2011). These observations confirm that the management of mixed and novel communities requires integrated schemes that are responsive to change and involve the continued monitoring of evolutionary processes (Cassini, 2020).

In an ever-changing world, with novel species being frequently incorporated into local communities, there is a strong need to broaden management approaches, focusing not only on the restoration of 'pristine' conditions but also including the development of future resilience, where native species can prosper under dynamic and flexible biotic regimes (Hansen, 2015). Although there is growing consensus about the broad application of management options promoting the persistence of native species in the presence of IAS (Adams & Pearl, 2007; Reise et al., 2017; Mumby et al., 2018), behavioural and evolutionary processes are still not integrated into conservation actions (Watters, Lema & Nevitt, 2003; Schlaepfer et al., 2005), which continue to stay focused on the removal of invaders from ecosystems. Even if steps have been made in the direction of 'conciliation biology' (Carroll, 2011), there is an urgent need for long-term studies to

elucidate complex ecosystem processes that include both native and non-native species (Cassini, 2020).

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All the authors contributed critically to the different phases of the research and to the writing of the paper. Also, all authors gave their final approval for publication. The authors report no potential conflicts of interest.

ORCID

Alessandra Maria Bissattini  <https://orcid.org/0000-0001-8575-1054>

Leonardo Vignoli  <https://orcid.org/0000-0002-1599-092X>

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SUPPORTING INFORMATION

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